Amygdala volume in a cognitively impaired population at enhanced risk of schizophrenia

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DECLARATION

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CONTRIBUTORS

This thesis has been composed using work undertaken as part of the Edinburgh Study of Comorbidity (ESC), hence a wide range of people have assisted with the data collection.

Professor E.C. Johnstone, Professor D.G.C. Owens, Dr P. Hoare, Dr W. Muir and Dr S. Lawrie conceived and designed the ESC; V. Moffat organised the recruitment of participants; Professor E.C. Johnstone and Professor D.G. Owens carried out the clinical assessments; Dr M. Spencer, Dr S. Gaur and Dr A. Stanfield were involved in the collection of structured rating scales; J. Harris and R. Krussenberg carried out IQ assessments and Dr A. Stanfield, R. Philip and Dr B. Moorhead were involved with collection of neuroimaging data. Dr A. Stanfield was involved in reliability studies and provided significant input with statistical issues.

I was involved in modification of the protocol for delineation of the amygdala, implemented this protocol, undertook the literature reviews and wrote the manuscript.

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ABSRACT

It is established that the mildly learning disabled population has a three fold elevated risk for schizophrenia. On the basis of findings from previous neuroimaging studies it has been proposed that in some individuals learning disability is a prepsychotic manifestation of schizophrenia. On this background, a cohort was selected from a nonpsychotic adolescent population in special education by employing tools previously shown to identify those at elevated risk of schizophrenia from within a high risk population. The risk of developing schizophrenia within this selected cohort was expected to be substantially greater than that for the learning disabled population as a whole. This population was then assessed by clinical interview, neuropsychological assessment and MRI scanning. Region of interest methodology was employed to ascertain amygdala volume in both the high risk and a matched control group. Two primary areas of interest were addressed; comparison of amygdala volume between the two groups and investigation of the relationship between symptomatology and amygdala volume within the high risk population. While no significant difference was found between amygdala volume in the high risk and control groups, a significant negative correlation was seen between left amygdala volume and weight of negative symptoms within the high risk group ($p=0.009$). This suggests that within this population reduced amygdala volume may be significant in the aetiology of negative-type symptoms and these symptoms may be present prior to clinical illness.

INDEX

Chapter 1

Introduction and Literature Review

1.1 Why the amygdala is of interest in schizophrenia

History of understanding of amygdala function

It has long been postulated that the amygdala may be a significant brain structure in the aetiology of schizophrenia. This mooting of the amygdala was initially proposed on the basis of theoretical considerations, but as evidence has accumulated this has been supported by experimental data.

When the evolution of understanding of amygdala function is considered, it is easy to see why it has drawn interest from researchers in schizophrenia. The experience of fear is a prominent feature of this condition,[1] and behavioural change is often associated; both of these phenomena have long been linked with amygdala function. In relation to the latter, the early work of Kluver and Bucy revealed that amygdala damage resulted in dramatic behavioural change.[2] Additionally, amygdalectomised monkeys appeared to no longer experience fear,[3] and as data has accumulated the importance of the amygdala in the experience of this emotion has been further supported. Michael Davis has reviewed the role of the amygdala in conditioned fear, anxiety and attention.[4] He summarises data supporting the idea that the amygdala, together with its many projections, may represent a central fear system involved in both the expression and acquisition of conditioned and unconditioned fear. This data includes findings from various animal species, acquired using disparate techniques. Some examples of this laboratory acquired data are that electrical stimulation and lesions of the amygdala respectively mimic or block natural and conditioned fear; local infusions of drugs such as flumazenil and morphine into the amygdala can have, respectively, anxiogenic and anxiolytic effects; and that

amygdala NMDA receptors are important in the acquisition of conditioned fear.[4] Additionally, fear and anxiety often precede temporal lobe epileptic seizures, which are usually associated with abnormal electrical activity of the amygdala. Given the centrality of fear to the experience of schizophrenic illness, on the basis of this data alone the potential significance of this structure is intriguing.

As understanding of amygdala function has expanded, its proposed role in attaching salience to stimuli and its importance in emotional memory have been intensively investigated. It is certainly a structure well placed for such a role. Rat studies have shown that it receives highly processed sensory information, primarily through its lateral and basolateral nuclei.[4] These nuclei then project to the central nucleus, which in turn projects to hypothalamic and brainstem areas that directly mediate specific signs of fear and anxiety. The central nucleus of the amygdala also has the potential for widespread indirect effects on the cortex via other projections.[4] In animal studies electrical stimulation of the amygdala appears to result in increased attention.[5]

The importance of the amygdala in conditioned fear is suggested by data such as that reporting that human subjects with amygdala lesions have deficits in classical fear conditioning using galvanic skin response as a measure.[6] Animal studies have linked specific lesions with specific deficits, with central nucleus lesions blocking the normal cardiovascular changes that develop during classical fear conditioning. For example, in rabbits, a cue paired with shock leads to bradycardia that can be blocked by either chemical or mechanical lesions of this region.[6,7,8] Other studies have shown that both NMDA-dependant and NMDA-independent long term potentiation can occur in both amygdala brain slices and in vivo[4] and that local infusion of an NMDA antagonist can prevent the acquisition of fear potentiated startle.[9] As well as aversive,

the amygdala appears also to be involved in appetitive associations; examples include association of visual cues with drug taking.[10] Overall however data suggests that the amygdala, like the hippocampal formation, plays a temporally limited role in memory processing, and is not the permanent memory storage site.[4] For avoidance-type responses as well as other forms of memory, it seems likely that the amygdala promotes memory storage in various non-amygdalar brain circuits, with this promotion dependant on the emotional significance of the events to be recorded.[11]

As described above, it has been shown that the amygdala is important in a variety of motivated behaviours in various species. It has been proposed that in humans however the amygdala has specialised to be particularly important in processing information relevant to the social domain, and is disproportionately important for social cognition and motivation of social behaviour.[12] One aspect of this is making judgements about the internal states of others based on expressed emotions. The role of the amygdala in the recognition of emotions of negative valence important to social interaction has long been recognised. For example, humans with bilateral amygdala damage have particular impairments in making judgements about such emotions, often with particular difficulties in recognition of fear.[12] While more recent studies have shown that the effect of amygdala damage on emotion recognition does show individual variation, with emotions other than fear being affected in some, the data does support it having a particular role in emotions of negative valence. Additional support for this role comes from functional studies, in normal individuals, which have shown amygdala activation to be particularly pronounced when looking at fearful faces.[13,14] The importance of the amygdala in the human social domain may however be broader still, going beyond simple emotion recognition, and extending to social judgements and 'theory of mind' abilities.

The role of the amygdala in social judgements was first suggested by studies in non-human primates, which demonstrated impaired social behaviour following amygdala damage.[15] More recent studies suggest a role for the amygdala in so-called 'theory of mind' abilities. This term encompasses the collection of abilities whereby we attribute internal mental states, intentions, desires, and emotions to other people. There is substantial data to support the idea that the amygdala plays an important role in it. For example, studies have shown that subjects with bilateral amygdala damage were specifically impaired in their ability to judge the untrustworthiness and inapproachability of people in photographs.[16] A further case report describes marked impairment of 'theory of mind' in the context of preserved executive function in an individual with unilateral, left sided amygdala damage.[17] Thus, they say, it seems highly likely that there is dissociation between these two spheres of ability. Additionally, functional imaging studies in normal individuals have shown increased amygdala activation when looking at untrustworthy rather than trustworthy photographs of people. A further study suggests an even broader role for the amygdala in making more general social attributions; individuals with amygdala damage were unable to make these based on the motion of visual stimuli, while unimpaired individuals found it difficult not to.[12]

Two other groups of researchers have investigated the role of the amygdala in social cognition through very different means. Intriguingly, Barton and Aggleton reported that there was a correlation between the volume of the basolateral amygdala, the size of the neocortex, and the size and complexity of social groups in 83 species of primates and insectivores.[18] A very different study, in humans, investigated if people with amygdala damage were particularly impaired in recognising 'social' emotions compared to more basic emotions; these are emotions such as jealousy, pride or

embarrassment, which necessarily require a social context. This was indeed found to be the case.[19]

If it is accepted that the amygdala is indeed important in motivated behaviour, how may it fulfil this role? One proposal is that it associates a representation of a stimulus with a representation of the emotional and social contingencies. This can thus result in modulation of behaviour. While this provides a hypothesis for the role of the amygdala in motivational behaviour however, it does not explain why it would have a disproportionate role in processing social stimuli and be central to social cognition. This is important, as the weight of evidence above does indeed suggest that the human amygdala has a disproportionate role in processing those stimuli (and possibly especially visual stimuli), that have an explicitly social significance.[12] Unfortunately this question is largely unanswered.

Relevance of understanding of amygdala function to schizophrenia

If it is the case that the amygdala has importance in attaching emotional salience to stimuli and particularly in processing social stimuli its potential significance in schizophrenia is clear. Studies have shown that patients with this condition do appear to show impairment in their ability to identify and accurately interpret emotions from sources such as facial expressions. They also exhibit deficits in emotion processing, emotional experience and behaviour with an apparent emphasis on the decoding and encoding of negative emotions.[20,21] Neutral stimuli are frequently imbued with great emotional significance which is often fearful, the emotion most closely associated with the amygdala. Impairments in emotion

perception and processing have been closely associated with the inability of many patients with schizophrenia to interpret various social cues appropriately and in proper context with respect to a given social situation. This can contribute to the formation of social cognitive biases, such as to judge others more negatively, as well as to conclude that others hold strong unfavourable impressions of them.[22] Interestingly, though patients free of positive symptoms show better performance in their ability to recognize and make social judgements from facial expressions, even those largely free of positive symptoms show deficits in social cognition.[23] Functional imaging studies have also shown abnormalities of emotional processing in individuals with schizophrenia. One such study involved functional imaging of a group of individuals with schizophrenia during an emotion recognition task. It showed that relative to healthy comparison participants, individuals with schizophrenia were not only less accurate in identifying emotions, but also displayed no amygdalar activation to fearful expressions.[24]

Given the deficits in emotion perception and processing described above, various hypotheses have been proposed to explain how amygdala dysfunction could be responsible for them. It has been suggested that patients with schizophrenia fail to activate their amygdala in response to sad, aversive or threatening stimuli, leading to failure to process fearful or threatening stimuli.[25] This could contribute to difficulty in recognition of some emotions. Conversely, neutral stimuli result in amygdala activation to a stronger extent than in normal subjects, leading to the aberrant assignment of salience to otherwise insignificant stimuli.[25] By the latter theory it can be seen how psychotic symptoms such as delusions of reference, and even primary delusions could occur. Indeed, there is some support for the above theories from functional imaging studies; Taylor et al found that activity in the left amygdala

correlated with positive symptoms (higher levels of symptoms associated with higher levels of activity), suggesting that amygdala activation may indeed be involved with positive symptom generation.[26]

While there has been much focus on the amygdala being important in the aetiology of positive symptoms, less attention has been paid to its potential role in negative symptoms. It is often argued that frontal lobe impairments are most likely the cause of these symptoms, as features such as psychomotor retardation, avolition and attentional impairment suggest deficits in this brain region. Further weight is given to this theory by findings such as smaller prefrontal white matter volumes being associated with severity of negative symptoms,[24] and patients with predominantly negative symptoms having lower levels of glucose metabolism in the prefrontal cortex compared to comparison subjects.[27] Given the weight of evidence for the importance of the amygdala in motivational behaviour and its role in processing social stimuli however, an argument can also be made for the potential involvement of the amygdala in this constellation of symptoms. Additionally, these brain regions are likely intimately related; in Brothers' seminal article proposing a neural system of social cognition for example, this system was composed of the amygdala as well as the superior temporal sulcus and orbitofrontal cortex.[28] On considering specific negative symptoms, an impairment in the drive for social interaction could clearly be significant in apathy and anhedonia, and decrease in emotional expression has potential links to defects in emotional recognition. Interestingly, and contrary to some findings (discussed below), if the amygdala was involved in such symptoms we might expect to see greater abnormalities in the right rather than left amygdala; this may be expected as there is some suggestion that the right side of the brain may be more involved in the identification and expression of emotion.[27]

On attempting to relate what is known about amygdala function to the social impairment that is such a significant consequence of negative symptoms, there is some evidence of an association. There is evidence, for example, that social dysfunction is predicted to a larger degree by impairments in facial affect recognition than by impairments in non-social cognitive functions such as memory, attention and executive functioning.[24] It seems logical to expect that apathy and avolition would be compounded by repeated ill judged attempts at social interaction which if based on an incorrect assessment of social information lead to further rejection and ultimately withdrawal.

Though amygdala involvement in negative symptoms is theoretically attractive, data supporting this is sparse. Potkin *et al* did find that only subjects with predominantly positive symptoms had increased glucose metabolism in the extended amygdala, but both the predominantly negative and positive symptom groups had higher levels of metabolism in portions of the amygdala compared to controls.[27]

1.2 Literature review of amygdala imaging studies

Introduction to imaging summary

Since Johnstone *et al*'s finding of increased ventricular size in people with schizophrenia,[29] it has been clear that there are structural abnormalities in the brains of this patient group. The most immediate challenge was to establish which brain structures were reduced to account for the increase in ventricular size. Unfortunately, initial studies investigating specific brain regions were hampered by the limitations inherent in the technology of the time. Since this seminal research however, imaging techniques have become more sensitive and brain structures easier to delineate and measure. This development of technology has been accompanied by continued interest in identifying structural abnormalities in brains of people with schizophrenia, with a wide variety of brain regions being targeted for investigation. Amongst these studies there has been particular interest in medial temporal lobe structures, with the hippocampus and amygdala receiving considerable attention. The reason for this interest is twofold: Firstly reduction in medial temporal lobe volume has been one of the more consistent findings in imaging studies.[30] Secondly, as described above, the overlap between the psychopathology of schizophrenia and what we know of the functions of these brain structures make them prime candidates for involvement in the disease process.

The accumulated data on brain imaging in schizophrenia is vast and disparate. Given that this report concerns the amygdala, I will limit this review to data concerning this structure. Until recent years imaging techniques were not of sufficient sensitivity to enable separate measurement of the amygdala and hippocampus; for this

reason studies focused on measurement of the amygdala-hippocampal complex (AHC). More recent studies, employing improved technology, have managed to differentiate the two structures. For reasons of completeness I will include the earlier studies which measured the amygdala and hippocampus together in this discussion. I will also, at times, refer to hippocampal measurements.

In vivo study of medial temporal lobe structures is a relatively recent endeavour. This is largely because such studies were impossible before the availability of MRI, which made possible the accurate separation of grey and white matter. Since the advent of this technology however, techniques employed in the measurement of these structure have progressively increased in sophistication. While initial studies involved hand tracing for example, over time this was superseded by semi-automated region of interest methodology. Indeed, though laborious, this remains the gold standard.[31] In recent years techniques have progressed further still, and there has been increasing interest in fully automated processes of regional measurement. One of the most prominent of the latter processes is voxel based morphometry (VBM). The variety of techniques all provide useful data and so, although this study involves region of interest methodology, these other techniques will also be included in the review.

On reviewing studies concerning amygdala volumetry, it is rapidly apparent that significant differences in methodology can exist between studies. This has potentially significant implications. One of the more important of these variables is differences in anatomical delineation, a point which will be expanded on later. A further issue is that in some studies whole brain volume is controlled for, while in others it is not. This is also potentially important. Though individual studies have failed to find a significant difference in brain volume between people with

schizophrenia and controls,[32] when studies are combined a reduction is seen.[33,34] A volume reduction of about 3% has been suggested, mainly attributable to loss of grey matter.[33] Whole brain volume reductions are particularly equivocal in first episode studies,[35,36] but it must still be born in mind that if studies have not controlled for whole brain volume, then any volume reductions which are seen could be negated by this approach. The methods employed in controlling for whole brain volume in imaging studies include either quoting structure volumes as a proportion of whole brain volume or covariation for whole brain volume.[37]

Review of studies investigating amygdala volume in schizophrenia

1.2.1 Findings in studies of chronic schizophrenia

It is well recognised that symptom development in schizophrenia is not static, and indeed that impairment associated with the condition tends to accrue over time. Given this, distinct populations of individuals have been investigated; patients with chronic schizophrenia, first episode patients and those at high risk of the condition. Additionally there have also been studies of first degree relatives of those with schizophrenia; individuals who obviously share genetic factors with effected individuals, but lack disease-specific differences. If neuroanatomical abnormalities are associated with the condition however, then if present in any group, they would be expected to be most marked in those with chronic schizophrenia. Unsurprisingly, it

was indeed in this group that ventricular enlargement was first observed. As imaging sensitivities improved, so amygdala size in those with schizophrenia could be investigated in comparison to controls. In a 1998 systematic review by Lawrie and Abukmeil they discuss six studies comparing volumes of the amygdala in those with schizophrenia with controls.[33] They include studies investigating both chronic and first episode cases published before June 1996. The nature of the studies meant a median evaluation of amygdala volume reduction could only be made for men, but this suggested a large and bilateral reduction of about 10%. The methodology did not control for whole brain volume. They felt the durability of this finding was supported by the fact that studies covering only the anterior part of the temporal lobe or AHC also reported large size reductions.

Since the review described above further studies have been published investigating the association between schizophrenia and amygdala size. Wright *et al* undertook a meta-analysis of structural imaging studies in schizophrenia involving volumetric brain MRI measurements made by region of interest technique published up to 1998, which again included chronic and first episode cases.[34] This review was based on seven studies that measured the amygdala as a distinct structure, volume not being adjusted for whole brain volume. It did show however that relative to the global reduction in cerebral volume, amygdala volumes were relatively small being 94% on the left and right compared to controls. A more recent review by Shenton *et al* includes studies published up to August 2000. Though meta-analysis is not attempted, the number of studies discussed is increased and they report that 74% of studies have positive findings compared to 26% negative. They discuss that evidence supports a volume reduction in the AHC compared to controls, though question whether these findings are specific to schizophrenia.[38] They also report that a

substantial number of studies demonstrate left-lateralized findings for AHC volume reductions, particularly in male patients.

Single studies of note include that of Gur *et al* in 2000, a relatively large study including 100 individuals with a mixture of first episode and chronic schizophrenia and 110 healthy controls.[39] This study was included in the review of Shenton *et al* discussed above. They reported decreased amygdala volume in men with schizophrenia (by 8%), but increased volume in women (by 10.5%). This dissociation between the sexes seems an isolated finding. A 2005 study by Suzuki *et al* employing ROI methodology to compare amygdala volume in people with schizophrenia, schizotypal disorder and controls found both right and left amygdala volume to be significantly reduced in schizophrenic subjects compared to controls.[40]

1.2.2 Review of findings in first episode studies

Early reviews commenting on amygdala size in schizophrenia had tended to include both chronic and first episode patients together. As studies accumulated however separate analysis of data relating to the two groups became possible. By the time of Shenton et al's review, above, data are listed separately for a number of such studies already undertaken. Data continued to accumulate however, and two meta-analyses of cross-sectional studies investigating brain abnormalities in first episode patients were published in 2006. Vita *et al*'s review looked at right and left amygdala separately. Their quantitative meta-analysis, involving a total of 115 patients and 88 controls, though finding a trend towards smaller amygdala in cases, greater in left than right, did not reach significance.[41] The methodology did not control for whole

brain volume. Interestingly they did find a significantly reduced hippocampal volume (left and right) leading to speculation that this structure may be affected earlier in the course of schizophrenia. The other recent meta-analysis, including several additional studies, was undertaken by Steen *et al*.[42] Unfortunately this meta-analysis did not include amygdala size, though it did find a statistically significant reduction in both right and left hippocampal volume of 8%.

For reasons that are not clear, one particular potentially interesting first episode study was not included in the meta-analysis of Vita *et al*. This is a study undertaken by Joyal *et al*, in which amygdala volume was determined in a group of medcation-naive patients with schizophrenia. As exposure to neuroleptics can so often be a potential confounding factor in studies of schizophrenia, though a small study (18 patients with schizophrenia and 22 controls), it is still worthy of discussion. It found, after controlling for intracranial area, gender, age and handedness, that individuals with schizophrenia had significantly lower amygdaloid volumes bilaterally.[43]

1.2.3 VBM studies in established schizophrenia

In addition to region of interest investigations, over recent years automated techniques of brain region measurement have also been developed. Prominent among these is the technique known as voxel based morphometry (VBM). As well as being less laborious, VBM also has the potential advantage of reducing any bias involved with user-dependant methods. In addition this technique looks at changes in every voxel in the brain, meaning that the region investigated is not constrained by prior

hypothesis. In their 2005 review of VBM studies, Honea *et al* report that volume deficits in the left amygdala are found in 'the majority' of studies reviewed.[44] This statement was made on a mixture of first episode and chronic schizophrenia patients. A notable single study included in this review was that that of Hulshoff Pol *et al*. They compared 159 patients to 158 healthy control subjects matched on age, sex, handedness and parental education. A significant reduction in grey matter density was reported for the left amygdala in the schizophrenia group, in addition to a number of other specific brain regions. The decreased density in the amygdala was more pronounced in older patients with schizophrenia. It was thus suggested that illness duration may be associated with greater amygdala volume reduction.[45] It must of course be acknowledged however that this study is potentially subject to selection bias, the older subjects having a more severe manifestation of the illness and this giving rise to the observed differences.

It is of note on reviewing VBM studies that it can be difficult with this technique to delineate the amygdala as a distinct structure, rather than brain region. It is also the case that it can be very difficult to detrend brain size effects from local measures, and though compensatory mechanisms can be employed, it is recognised that brain size can confound with this technique.[46]

1.2.4 Longitudinal studies

The above data suggest that changes in the brains of those with first episode schizophrenia may be less marked than in those in the chronic stage of the illness. If this was the case, it would suggest that there may be progressive brain changes over

time. Evidence for this would clearly be stronger however if changes were shown to occur in a cohort with schizophrenia followed up over time, but not in a control group. Unfortunately however there are numerous practical difficulties in conducting studies such as these. Most obvious is that of subject retention. This is made even more problematic by the fact that brain volume loss over a year may be close to the limit of detection by MRI, necessitating lengthy studies.[42] Actually being able to undertake comparable scans however can be an even greater problem, a concept known as 'machine drift'.[42] MRI machines are often not comparable even if the same model, and obviously in longitudinal studies by the time of the second scan the original machine may be obsolete. A different machine at the second timepoint can thus introduce systematic bias. Even if he same machine is used, positioning for the second scan may be different and produce distortion.

Despite the practical difficulties detailed above, longitudinal studies have been carried out from the early 1990s. One such MRI study by DeLisi and colleagues achieved follow up of 27 first-episode patients and 10 controls over ten years. While progressive ventricular enlargement of cases was shown, progressively decreasing volume of the AHC was not.[47] As well as the problem of scanner comparability over time, this is an early study with older equipment and thick slices; a relatively large interslice gap of 2mm was employed. This reduces sensitivity in finding small differences still further. Since this early study numerous other longitudinal studies have been undertaken; equipment employed has been more sensitive, but follow up periods often shorter. These longitudinal studies were summarised by Pantelis *et al* in 2005.[48] Findings from these other studies have been inconsistent and at times surprising. Gur *et al* for example, in their study comparing first episode, established schizophrenia cases and controls found that while temporal lobe volume reduction

over time was greater in first episode than established cases, it was greatest of all in controls.[49]

Only some of the studies reviewed by Pantelis *et al* specifically measure the AHC, and fewer still separately measure the amygdala. Among those that do the former, Kasai *et al*'s study of thirteen first episode subjects followed up over an average of 1.43 years did not demonstrate a reduction in size of the AHC.[50] James *et al*'s study was in an adolescent onset, not adult group, but did separate hippocampus from amygdala; while the average size of the left amygdale of the 16 in the study group was 12% smaller in males and 7% smaller in females compared to controls, further reductions were not demonstrated in the average 2.7 years follow-up period.[51]

A literature search did reveal one more recent longitudinal volumetric study, not included in Pantelis *et al'*s review, in which attempts were made to measure the amygdala as a separate structure. In this study, by Whitworth *et al*, 21 first episode and 17 multiple episode schizophrenia patients and 20 healthy controls were followed up for between two to four years.[52] They thought accurate separation of the amygdala and hippocampus was not possible and so measured it as a single structure which was then divided into an anterior (amygdalar) and posterior (hippocampal) portion. Adjustments were made for whole brain volume. Though there was a non-significant trend for smaller amygdala in patients compared to controls at both time points, there was no evidence for reduction in amygdala size in either patient group over time.

Overall these data suggest that if amygdala volume reductions do occur once schizophrenia has become clinically manifest, then they are small. They do however add to the weight of data suggesting that at the point of first episode of psychosis the amygdalas of those with schizophrenia are smaller than those of controls.

1.2.5 High risk studies

As discussed above, there is little evidence for progressive reduction in amygdala size once schizophrenia has been diagnosed. Even if this evidence was present however, with so many confounding factors such as exposure to medication and lifestyle, this would not necessarily mean that these changes are due to schizophrenia itself. What would be more convincing in illustrating that any reductions in amygdala size were due to the process of the illness would be if brain changes were shown to correlate with the development of symptoms before medication is commenced. Additionally, it would be of significant potential utility if it could be demonstrated that the brains of those who are destined to develop schizophrenia are different from those who are not, even before the disease has become manifest. Investigation of this possibility would require the identification and imaging of subjects prior to the development of psychotic symptoms and follow up with repeated investigation during the time they do or do not develop schizophrenia. The need for such studies has long been recognised, but they are notoriously difficult to conduct. These difficulties are similar to those described for all longitudinal studies. Additionally however, as not all subjects under investigation will develop schizophrenia, to achieve adequate numbers who do cohorts must be large. This magnifies difficulties such as subject retention and identification of appropriate controls. Various approaches have been adopted by different research groups to circumvent these difficulties; I will focus on the findings from two of the most high profile of these groups, the Edinburgh and Melbourne groups, but also mention other relevant findings.

The Melbourne group used a 'close in' strategy to identify those symptomatic, clinically compromised, and help-seeking individuals at imminent risk of developing a florid psychosis, but not yet actually psychotic. They expected 30-40% of this ultra-high risk (UHR) group to make the transition to florid psychosis. Their biggest study published to date consisted of 135 patients identified as UHR of whom 39 developed psychosis (UHR-P) and 96 did not (UHR-NP).[53] The characteristics of these groups were compared to 162 first-episode patients (a mixture of schizophrenia, schizoaffective, affective and other psychosis), 89 chronic schizophrenics and 87 normal controls, with measurement of amygdala volume (by ROI methodology) being among those criteria investigated. While left hippocampi were of normal size in UHR subjects, and reduced in schizophrenic subjects (both first episode and chronic), this study found increased amygdala size in affective psychosis (a finding previously reported),[54] or psychosis not otherwise specified, but normal amygdala size in subjects with schizophrenia and UHR subjects. Though longitudinal results from this large cohort have not been published, there have been reports from a smaller group of UHR individuals who did develop psychosis.[55] In this study 75 UHR subjects where identified of whom 23 had developed psychosis after 'at least' 12 months. Ten who had developed psychosis and 11 who had not were rescanned after this time period and the scans compared by VBM. Though the amygdala is not specifically mentioned they did find that there were smaller volumes of grey matter in the temporal lobes at the time of first scan in those of the UHR group who went on to develop psychosis compared to those who did not, and that further reductions occurred in this group between the two timepoints. Longitudinal findings from the larger cohort are awaited.

The Edinburgh group recruited at an earlier stage still; before those who could be destined to develop psychosis had made any attempts to seek help. Subjects were

defined as being high risk by virtue of having two affected relatives. They were identified in adolescence before clear manifestation of schizophrenia, assessed at this point and followed up for ten years. This was achieved, in brief, by identifying from among the population of individuals with schizophrenia, those who had both a family history of the condition and adolescent relatives. Diagnosis of schizophrenia was verified, relatives of the individuals with schizophrenia approached, and high-risk subjects aged 16-25 who agreed to participate given a detailed clinical, neuropsychological and brain imaging assessment. These assessments were then repeated after approximately 2 years in consenting participants. Two control groups, one consisting of individuals without a family history of schizophrenia and one of first-episode schizophrenics were also recruited. These groups were subject to the same investigations as described above.

Results from the Edinburgh High Risk Study (EHRS) have now been published. The findings at the start of the study are themselves interesting. At baseline, ROI analysis found that the mean volumes of the left and right AHCs in high risk subjects were significantly smaller than those in controls (about 4%), but larger than those in first-episode schizophrenics (by about 4%).[56] Whole brain volume, together with other factors such as sex and age were controlled for. This finding suggests that abnormalities in this region may indeed pre-date the onset of psychosis. On both sides this result was highly significant, (<0.01), but significance was greater on the left. On analysis of scans with automated VBM overall findings were similar to those by ROI methodology. It is the case however that though amygdala volume reductions were seen on comparing first-episode to high risk subjects by VBM, these were not present comparing high risk and controls.[57] Though this is surprising, it is

recognised that some differences can exist in findings from the two methodologies, and ROI techniques are regarded as the gold standard.[31]

There is also evidence of brain structural changes as psychotic symptoms develop over time. In their 2002 paper Lawrie *et al* discuss their finding that within a high risk group of 66 patients, the 19 with transient or isolated psychotic symptoms (12 of whom had these at the point of first assessment, of importance these symptoms were not of sufficient severity to justify a diagnosis of schizophrenia) display a statistically significant greater volume reduction in the right temporal lobe when compared to those without psychotic symptoms over approximately two years.[58] A reduction was also seen in the left temporal lobe, though this did not reach statistical significance. Amygdala-hippocampal complex measurements were also repeated by ROI methods at this timepoint; though no significant volume reduction was seen in this structure, it is suggested that the measurement error inherent in this method may have obscured a reduction which did occur.[56] The fact that only 66 of the high risk subjects could be included in this analysis is worthy of mention and underlines one of the difficulties with longitudinal studies raised above. In this study the scanning machine was changed halfway through the study and so only 66 high risk subjects, those recruited earliest into the study, had both initial and follow up scans on the same machine. As a second scan with a different machine could introduce systematic bias, only those subjects with scans on the same machine, with current methodologies at least, are appropriate for comparison.

As mentioned above, in addition to ROI analysis the EHRS scans were also analysed with VBM. This was used to both investigate baseline differences between the three groups and changes within the groups over the two years between the first and second scans. Particular attention was paid to the amygdala-hippocampal

complex. By the end of the EHRS 21 high risk subjects had developed schizophrenia, often some years after the second scan.[60] Of these 21, 8 had both the initial and follow up scans on the same machine; this was again due to the scanner being changed midway through the study. By VBM methodology, reduction over time in a variety of left temporal lobe structures (rather than being more pronounced in the right temporal lobe, as in the ROI study) was seen in high risk subjects with transient or isolated psychotic symptoms. Additionally, this group showed reductions in the right amygdala, but these latter changes were not sustained when significant changes in those high risk subjects without psychotic symptoms were excluded. In those eight subjects who had developed schizophrenia by 2003 however, grey matter density reductions were seen in the left temporal lobe in baseline scans when compared to controls; over time further volume reductions were seen in the left uncus, statistically significant after making an amygdalo-hippocampal volume correction designed to encompass the whole amygdale and hippocampus.[60]

Relevant findings in other high risk populations

Further interesting findings in a high risk population have been published from a population in Pittsburgh.[61] This study investigated brain volumes in unaffected, young offspring of schizophrenia patients, individuals at high risk of developing schizophrenia. This group measured amygdala and hippocampus together and reported reduced volumes of the left anterior and posterior AHC, after adjusting for intracranial volume. The anterior AHC can be considered to correspond roughly to the amygdala. The authors suggest these abnormalities to be of neurodevelopmental origin. Of note, reductions in volume of the dorsolateral pre-frontal cortex (DLPFC) were not seen in this population, leading to speculation that these may occur later than

AHC changes, a possibility they related to the DLPFC being the last part of the brain to mature.

1.2.6 Functional imaging studies investigating the role of the amygdala in schizophrenia

Functional imaging studies have also been employed to investigate abnormalities in the brains of people with schizophrenia. Findings of abnormalities of amygdala activation in patients with schizophrenia are discussed by Aleman and Kahn in their 2005 review.[25] Studies reported show lack of amygdala activation during negative affect[62] and lack of amygdala activation in response to fearful expressions.[63] A study by Taylor *et al* demonstrated less activation of the right amygdala in subjects with schizophrenia compared to controls, together with increased activity in the left amygdala of patients correlating with higher levels of positive symptoms.[26] From the latter data it is discussed that reduced amygdala activity may underlie deficits in emotion recognition, while activation may be involved in positive symptoms. Overall however the conclusions of this review are that the most consistent picture to emerge from functional neuroimaging studies in schizophrenia is of reduced activation of the amygdala in response to emotional stimuli compared to neutral stimuli. It may well be that this is coupled to stronger activation of the amygdala in response to neutral stimuli than is the case in controls; this, of course, would fit with a model of aberrant assignment of salience.

To identify studies addressing abnormalities of amygdala activation in schizophrenia published since Aleman and Kahn's review a literature search was

undertaken. Search terms were 'schizophrenia' combined using the 'AND' operator with 'imaging' and 'amygdala'. The search was run on EMBASE, PsychINFO and Medline. Relevant papers are summarised in Table 1.1 below.

As can be seen from Table 1.1, findings are at times somewhat contradictory and remain inconclusive; this is likely at least partially due to the heterogeneity of the groups investigated. For this reason particularly the paper of Fahim *et al* is interesting, as it does attempt to subdivide the schizophrenia group.[64] Also of note is the Holt *et al* study, which reported elevated activation of the right amygdala during neutral as well as fearful face observations;[67] this is at odds with the findings summarised by Aleman and Kahn.[25] This discrepancy may of course be due to group, medication and subtle task differences. If these possibilities are left aside however, this finding does contradict the theory that a lesion of the amygdala is associated with a reduction in neural response to emotional stimuli and consequent inability to process other peoples' emotions. Instead findings could be interpreted to suggest a more general dysregulation of the amygdalar response to social stimuli, in which interplay with regions such as the anterior prefrontal cortex is likely significant. Indeed, in-keeping with this, there is substantial evidence demonstrating the importance of prefrontal modulation on amygdalar activity.[70]

Study	Comparison groups	Relevant findings	Possible significance of findings
Fahim et al[64]	fMRI comparison of subjects with schizophrenia with (13) and without (11) flat affect viewing emotionally negative pictures	Significantly elevated brain activity in the lingual gyrus in group without flat affect only	Speculate that in flat affect amygdala malfunction renders amygdala unable to correctly evaluate the emotional meaning of pictures presented and hence link to parts of brain implicated in physiological and experiential dimensions of emotion.
Laurens et al[65]	fMRI comparison of subjects with schizophrenia (28) and healthy controls (28) exposed to task-irrelevant, infrequent novel stimuli	Patients with schizophrenia display relative underactivity during novel stimulus processing in right amygdala-hippcampus (together with numerous other regions)	Suggest that patients less effectively reorient processing resources away from the ongoing task, and experience increased distraction by novel stimuli. Believe this may indicate decreased efficiency of information processing.
Das et al[66]	fMRI comparison of subjects with schizophrenia (14) with controls (14) on exposure to fearful and neutral facial expression stimuli	Schizophrenia group display reduced amygdala activity in response to fearful facial expression and abnormal patterns of connectivity with the brainstem, visual cortex and regions of the MPFC	Indicate that sensory processing of potential threat is disrupted in schizophrenia. Breakdown of connectivity may limit the degree to which regions such as the amygdala are effectively engaged during fear processing. May contribute to a cycle of misattribution about incoming signals of potential threat in schizophrenia.
Holt et al[67]	fMRI comparison of subjects with schizophrenia (15) and control subjects (16) viewing faces displaying fearful, happy and neutral facial expressions	Schizophrenia group demonstrate increased right amygdala activation during the initial presentation of fearful and neutral facial expressions, and greater hippocampal activation on viewing all three.	Elevated MTL responses to neutral facial expressions compatible with a bias towards assigning emotional meaning to neutral information (aberrant assignment of emotional salience).
Sanjuan et al[68]	fMRI comparison of subjects with schizophrenia (11) and control subjects (10) exposed to neutral and emotional words	Enhanced activity of frontal lobe, temporal lobe, insula, cingulate and amygdala (mainly right side) in subjects hearing emotional words. No clear activation in response to neutral stimuli.	Acknowledge that majority of previous studies using emotional paradigms in schizophrenia show reduced activation in response to emotional stimuli. Suggest the difference is due to the fact that they used visual stimuli, which are less relevant to the symptom profile of schizophrenia

Table 1.1
Studies employing fMRI to investigate role of the amygdala in schizophrenia published after review of Aleman et al.

Abbreviations: MPFC, Medial Prefrontal Cortex; MTL, Medial Temporal Lobe.

1.3 Structural MRI studies in childhood-onset schizophrenia

Data from children/adolescents has at times been mentioned above, but it is important that this is expanded on. Though a rare condition, there is substantial data, and one large longitudinal study, on childhood-onset schizophrenia. This is defined as onset of psychotic symptoms before the thirteenth birthday. Subjects with this condition have more severe premorbid symptoms and a more chronic and severe course than later onset individuals.[70] Though the childhood age group is largely beyond the scope of this study, the fact that manifestations can be so severe in children makes it interesting, as anatomical findings might be expected to be more pronounced than in adults.

The biggest study of childhood onset schizophrenia is that of the National Institute of Mental Health (NIMH). Initial MRI results demonstrated no significant differences in amygdala volumes in effected subjects compared to controls (mean age 14.6 +/- 1.7 years.[71] Prospective longitudinal brain MRI rescan measures from the NIMH childhood onset schizophrenia sample show progressive abnormalities. These include decreasing temporal lobe volumes 2, 4 and 6 years after the initial scan. At two years, across mean ages 14 to 16 years, though a greater decrease in amygdala volume was seen in schizophrenic compared to control subjects, this did not persist after adjustment for total cerebral volume.[72] When this cohort was followed up at 4 years post initial scan no evidence for amygdala volume change with age was found for either the study or control group.[72]

A more recent study of childhood-onset schizophrenia, not based on the NIMH data, has produced rather more surprising results. This study, by Levitt *et al* compared thirteen children with schizophrenia (mean age 14.2 +/- 3.8 years), and 20

normal children.[70] After adjustment for age and total brain volume, the amygdala was larger in the schizophrenic than in the control subjects, and this volume increase was more pronounced on the left side. It must of course be remembered that this is a small study and a single result.

Other studies have examined amygdala volume in subjects with adolescent-onset schizophrenia. This data is included with the general discussion of adult data where appropriate.

1.4 Post mortem studies

As discussed above, imaging techniques have been widely employed to investigate structural abnormalities in the brains of living subjects with schizophrenia. For structures such as the amygdala, accurate delineation has only been possible following the development of sophisticated technologies such as MRI. Post mortem studies require no such technologies however, and the history of their use in the investigation of neuroanatomical abnormalities in schizophrenia has a much longer heritage. If this heritage is to be discussed however, the history of the concept of schizophrenia must be appreciated. This is generally considered to have been first defined as dementia precox by Emil Kraeplin in 1896, when he grouped together conditions such as hebephrenia and catatonia as manifestations of the same disorder, which typically had its onset in early adult life and had a poor outcome.[74]

Early studies investigating coarse brain structure were reviewed by Brown *et al.*[75] The earliest relevant study they identified compared brain weights from a group of subjects who had had a condition analogous to schizophrenia with those from

subjects who had had other psychiatric conditions. This was undertaken by Crichton-Brown, dates from 1879, and found brains from subjects with schizophrenia to be lighter than, for example, those from subjects who had had an affective disorder. Other studies in this review, which date up to the 1960s, generally revealed a tendency for schizophrenic subjects to have lower brain weights. It is of note however that in a number of studies there are problems concerning the use of control subjects with dementing conditions.[74]

Histological studies were carried out from the late nineteenth century, and though several did report abnormalities in the brains of people with schizophrenia, there was little consistency among these reports.[74] In 1924, Dunlap conducted a careful comparison of the brains of eight schizophrenic subjects who died at less than 45 years of age and five controls, selected in each case for a cause of death that was not likely to have influenced the structures of the brain. No difference in cell counts in the cerebral cortex were found between subjects and controls. This cast scepticism over the histopathological literature, but some work did continue, and evidence of gliosis and changes in the orientation of cells has been found.[74]

The first identifiable post-mortem study specifically investigating amygdala size dates from 1985. Though it did show reduced amydala volume,[76] this was not reported in several subsequent studies.[77,78] The most recent post-mortem study was undertaken by Chance *et al* and published in 2002.[79] They ascertained amygdala volume in the brains of 18 patients who had been diagnosed with schizophrenia compared to 18 controls. Clearly this study was limited by the use of elderly, medicated subjects, but the advantage of this investigatory technique is that delineation of the amygdala could occur at a histological level. In this small study they did not find differences in amygdala size between cases and controls, but as they

themselves discuss the study only had a 15% power to detect a change of 5-7% of amygdala volume. The limitations of post mortem studies must not be forgotten when interpreting this data. Obviously this includes potential factors such as differences in time from death to tissue fixation between cases and controls and tissue shrinkage. The most important limitation however is inherent in the nature of the population which must of necessity be studied. As noted above they are elderly subjects. As well as decades of exposure to neuroleptic medication, they will have been ill for many years, and had low levels of physical and mental activity. They, together with the control group are likely to have suffered an episode of severe physical ill health prior to death. Thus, even in the case of negative findings on comparing cases to controls, the degree to which this refutes that they occur as a consequence of schizophrenia itself is questionable.

1.6 Synthesis of data addressing abnormalities of the amygdala in schizophrenia

On reviewing the above it is clear that some of the evidence for amygdala abnormalities in schizophrenia is conflicting. The absence of differences between patients with schizophrenia and controls in the most recent post-mortem study, though potentially explicable by subject choice and other factors, is still surprising. Overall however, there is much evidence to suggest that amygdala abnormalities are present in the brains of schizophrenics. In addition, there is at least a suggestion, from the few pre-illness studies, that these changes may precede illness onset. There is no particular suggestion that the methodological variant of whether or not whole brain

volume is controlled for has a significant association with positive or negative findings.

A further factor apparent from the data above is the suggestion that in schizophrenia the left amygdala may exhibit greater or at least earlier volume reduction than the right. Of note, in the Vita *et al* meta-analysis of first episode studies the non-significant trend towards smaller amygdala was greater on the left than right side.[41] Additionally, in the Honea *et al* VBM review, which included a mixture of first episode and chronic cases, left sided amygdala volume reduction was reported to be found in 'the majority' of studies, with no mention of comparable reductions on the right side.[44] In the study of James *et al* once again specifically smaller amygdala volumes were seen at time of illness onset only on the left side,[51] while VBM analysis of subjects from the EHRS who developed schizophrenia suggested that volume loss was more marked on the left side.[60] In short, though bilateral volume reduction of the amygdala is seen in some studies and meta-analyses, when present only unilaterally, this is generally on the left side.

1.7 Review of studies investigating amygdala volume in relatives of those with schizophrenia

Twin and family studies

A family history of schizophrenia has long been established as one of the strongest risk factors for the disorder in an unaffected proband.[80] It is also well recognised that schizophrenia is a highly heritable condition, and that brain volumes themselves are highly heritable.[81] Given these factors it is thus clearly understandable why the examination of familial and genetic structural MRI associations has been a further area of investigation. I will summarise below those twin and family studies which have relevance to the elucidation of amygdala abnormalities in schizophrenia

Twin studies

Studies of monozygotic twins discordant for schizophrenia have the potential to distinguish environmental from genetic effects. Though there are important caveats,[82] greater neuroanatomical similarities would be presumed to reflect common genetic effects and differences to reflect environmental effects.

Though few in number, there are studies comparing amygdala volume in monozygotic twins discordant for schizophrenia. One such example is the study of Baare *et al*, which attempted delineation of the amygdala separate from the hippocampus.[83] It included 15 pairs of discordant monozygotic twins, 14 pairs of discordant dizygotic twins and 29 healthy twin pairs. Unfortunately, due to low intrarater reliability, the amygdala data was excluded from analysis. It was found however that irrespective of zygosity, affected co-twins had smaller hippocampal

volumes than their healthy co-twin.[83] Two more recent twin studies, though they did investigate hippocampal volume, once again did not address amygdala volume.[84,85] The study of van Erp *et al* again found hippocampal volume reduced in the individual with schizophrenia in both monozygotic and dizygotic pairs.[85] On the basis of their analysis they suggest that although hippocampal volumes in healthy twins are highly heritable, those in twins discordant for schizophrenia are subject to substantially greater modulation by environmental factors. It is surprising that no twin studies could be identified in which amygdala volume is investigated. Investigation of the genetic contribution to amygdala volume by these means has significant potential to yield useful data.

Family studies

The underlying rationale for investigating the first-degree relatives of patients with schizophrenia is that they share approximately 50% of their genome. As a consequence common differences versus controls probably reflect genetic factors, while differences between unaffected and affected relatives presumably represent disease-specific effects. There are a number of studies investigating the amygdala-hippocampal complex, and also some specifically addressing amygdala volume. Of the latter, that of Staal *et al* is important to discuss.[86] It employed region of interest methodology to compare volumes of various structure in 32 same-sex siblings discordant for schizophrenia and 32 matched controls. Amygdala volume (together with hippocampal) did not significantly differ between patients, siblings and comparison subjects.[86] This is in contrast to the study of Keshavan *et al*, again specifically measuring the amygdala, in which volume was reduced in first-degree relatives of those with schizophrenia compared to controls.[87] A number of studies

measuring the AHC have also found it reduced in relatives of those with schizophrenia compared to controls.[88,89] There is also evidence for smaller AHC in those with schizophrenia compared to well relatives.[90]

A refinement of family studies are those focusing on obligate carriers. The rationale for these arises from the fact that although the risk for relatives of developing schizophrenia is increased, only a subgroup will actually carry the pathological genes. Thus in multiply affected families affected subjects, non-obligate carriers (unaffected, with affected parent but no affected offspring) and obligate carriers (unaffected, with affected parent and child) can be identified. Such a design allows a clear separation of the contribution of pathological genes from the effects of the disease itself. In the EHRS AHC volumes (right and left combined) in subjects with schizophrenia and obligate carriers were significantly smaller when compared to non-affected/non-carriers, which implies a genetic contribution in AHC pathology in schizophrenia.[92] These results would suggest that some of the AHC abnormalities seen reflect an underlying genetic liability for schizophrenia, rather than being actual expression of the illness.

1.8 The amygdala in schizotypal disorder

As can be seen above, imaging studies focusing on the amygdala in schizophrenia have been numerous. They have included those focusing on subjects at high risk before illness onset, first episode subjects and relatives of affected individuals. All of these approaches should reduce the potential of confounding factors such as lifestyle and exposure to medication giving rise to any differences seen. It is recognised however that there is a further group of individuals who are of interest to the investigation of biological markers associated with schizophrenia. These are people with conditions which, due to their commonality with schizophrenia, have been grouped as the "schizophrenia spectrum disorders".

Foremost among these disorders is schizotypal personality disorder. This condition, similarly to schizophrenia, is characterized by positive or psychotic-like symptoms and negative or deficit-like symptoms. The positive-like symptoms include ideas of reference, cognitive or perceptual distortions, and magical thinking. Negative symptoms encompass social deficit and interpersonal difficulties. Cognitive disorganisation is also seen.[92] As well as symptom similarity there is also evidence of a genetic association between the two disorders, with a greater prevalence of schizotypal disorder being found in the relatives of those with schizophrenia, and also psychophysiological correlates between the two conditions.[92]

Structural imaging findings in the medial temporal regions of individuals with schizotypal disorder were reviewed in Siever et al's American Journal of Psychiatry review of 2004.[92] They note that though some temporal lobe structures have been found to be reduced in schizotypal subjects compared to controls, this reduction was

not seen in the medial temporal lobe, and in one, albeit small adult study, no difference was seen in the specifically measured amygdala.[93] In contrast to these results, a Japanese study comparing 59 controls, 25 schizotypals and 59 schizophrenics found that both amygdala and hippocampal volumes were reduced bilaterally in both subjects with schizophrenia and schizotypy compared to controls.[40] A VBM study by Kawasaki *et al* in the same centre, and with considerable overlap of subjects, compared 25 subjects with schizophrenia, 25 schizotypal subjects and 50 controls.[94] Patients with schizotypal disorder again showed grey matter reductions in the medial temporal region compared to controls. It should be noted however that in these studies schizotypal patients were recruited from already being in contact with services and the majority were receiving medication, and thus somewhat different from other schizotypal groups. Despite this limitations however, there are additional interesting findings from these studies. Not least of these is data from the Suzuki study concerning scores on the Scale for the Assessment of Negative Symptoms (SANS) and prefrontal cortex volumes. Though prefrontal cortex subcomponent volumes were reduced in those with schizophrenia, this was generally not the case in schizotypal subjects. This was despite the fact that schizotypal subjects scored as highly as schizophrenic subjects on the SANS. This does not seem compatible with the widely accepted belief that prefrontal cortical volume loss is associated with negative symptoms in schizophrenia.

Functional studies also suggest differences between patients with schizotypy and controls. Similar to schizophrenic patients, Mohanty *et al* report increased activity in the hippocampal and amygdalar regions during performance on an emotional Stroop test in patients with positive schizotypy compared to controls.[95]

A further means of investigating the relationship between schizotypal symptoms and schizophrenia is to establish the prevalence of these symptoms in individuals at high risk for schizophrenia. This was done in the EHRS, in which it was determined that higher scores on the Structured Interview for Schizotypy (SIS)[96] indicated increased risk of developing schizophrenia.[97] As part of this study the relationship between high scores on measures of schizotypy and neuroanatomical abnormalities, including those of the AHC was also investigated. Analysis of the high risk group within the EHRS failed to show a clear correlation between score on the SIS or Rust Inventory of Schizotypal Cognitions (RISC)[98] scores and AHC size.[99]

Implications of findings relating score on measures of schizotypy to vulnerability to developing schizophrenia

As mentioned above, within the high risk group of the EHRS, those scoring higher on the SIS were at elevated risk of developing schizophrenia, with certain factors, such as oddness and social withdrawal having particular predictive power.[97] Higher scores on the CBCL, a scale measuring childhood behavioural disturbance also predicted greater likelihood of developing schizophrenia.[97] This means that by employing these tools, from within a group already at elevated risk of developing schizophrenia, those whose risk is elevated further still can be identified. As will be seen below, this finding is fundamental to the design of the current study.

1.9 Schizophrenia and learning disability

1.9.1 Nature of the association

One of the aims of the EHRS, discussed in some detail above, was to try to establish if there was a potential way to identify individuals who may go on to develop schizophrenia. This would clearly have great utility, as preventative interventions could then be targeted to this group. In the EHRS the group targeted for study were those with an increased risk of developing schizophrenia by virtue of their family history. A further group known to be at elevated risk of the condition, but little studied as a high risk population, is those with learning disability.

It is acknowledged that at all stages of schizophrenia, premorbid, pre-illness, acute and chronic illness there is a relationship between schizophrenia and impairments in cognitive ability.[100] It is also well recognised that in people with mild learning disability (LD) there is an increased prevalence of schizophrenia. Within this group the point prevalence is 3%, three times that of the general population.[101] This acknowledgement of an association between schizophrenia and learning disability is not new. In 1919 Kraepelin described 'propfschizophrenie', a condition in which dementia praecox arose in a setting of pre-existing intellectual impairment.[102] He stated that dementia praecox could be diagnosed in 7% of people with learning disability, that the psychosis had a very early onset and that the pathological process involved in this actually led to the intellectual disability.

The association between schizophrenia and learning disability could of course result from various possibilities. The direction of association must either be however

the presence of schizophrenia (diagnosed or destined to develop) increasing the likelihood of an individual being diagnosed as having a learning disability or the presence of a learning disability increasing the likelihood of an individual being diagnosed as having schizophrenia. The latter possibility implies that the cognitive deficits of some individuals with learning disability convey an increased susceptibility to develop the impairments associated with schizophrenia, the suggested mechanism being the overload on comprehension imparted by partly understood stimuli.[103] The former possibility is consistent with the view that cognitive dysfunction, and sometimes motor and social impairment, in children may be the initial symptoms of severe schizophrenia.

In an attempt to clarify which of the above possibilities was most likely, studies were undertaken comparing subjects with co-morbid schizophrenia and learning disability to individuals with learning disability alone and schizophrenia alone in terms of clinical, imaging and genetic parameters.[103,104] Structural brain changes in the co-morbid sample were reported to strongly resemble those of the schizophrenia sample and be very different from the group with learning disability alone. One such commonality was the finding of smaller AHCs relative to whole brain size. Whole brain volume was controlled for in this study, and indeed while similar in the comorbid and schizophrenic groups, differed significantly from those in the learning disabled group. On the basis of the above and other data, it was postulated that co-morbidity represents a severe form of schizophrenia. This opens the possibility that within the young learning disabled population there may be individuals whose cognitive difficulties are part of the natural history of an illness where the clinical features that define schizophrenia have yet to become manifest.

1.9.2 Clarification of clinical features of schizophrenia in learning disability

In Doody *et al*'s paper, comparing schizophrenia in those with mild learning disability to that in those with normal IQ, a number of clinical differences were noted between the two groups.[103] Among these was a significant difference in rating on the negative subset of the Positive and Negative Symptom Scale (PANSS). Though the comorbid group showed no differences in positive, general or total symptom profiles compared with the schizophrenic control group, they did have significantly more negative symptoms. This is an intriguing finding, particularly in the context of co-occurrence of schizophrenia and learning disability being proposed as a specific manifestation of a severe form of schizophrenia. Given this I undertook a systematic review of psychopathology in schizophrenia associated with mild or borderline learning disability.

Literature search

Medline, EMBASE and PsychINFO were searched for all English language studies published between January 1984 and March 2007 that compared clinical features of schizophrenia in people with learning disability or borderline intellectual impairment and those with normal IQ. The search terms were "schizophrenia" combined using the AND operator with "learning disability", "mental retardation", or "intellectual impairment", AND "symptoms", or "psychopathology". Both free-text and expanded medical subject headings were used. Subject headings were adapted to the specific subject headings of the biomedical databases used. The search strategy was supplemented by inspecting the reference lists of included articles.

Criteria for inclusion

Abstracts were assessed for inclusion by the author, and articles in full text were retrieved if appropriate. Primary research studies were considered for inclusion if they were published as a peer-reviewed article in English and compared learning disabled or borderline learning disability subjects with schizophrenia with those of normal IQ. Additionally, they required to use a recognised symptom rating scale (i.e. BPRS, PANSS, SANS) to quantify symptoms including the negative subset.

Results

108 articles were identified through the database search. Only five of these, one the Doody paper, were suitable for inclusion in this review. These are shown in Table 1.2.

Study (year)	Study group	Learning disabled/ borderline intellectual impairment and schizophrennia			Schizophrenia without intellectual impairment			Main findings
		N	Mean/ median age	Mean/ median illness duration (years)	N	Mean/ median age	Mean/ median illness duration (years)	
Meadows (1991)[105]	Subjects comorbid for mild mental retardation and schizophrenia	25	35.4	12.9	26	43.2	17.0	Similar clinical presentation in both groups
Doody (1998)[103]	Subjects comorbid for mild learning disability and schizophrenia	39	48.6	4.9	34	48.6	5.0	Comorbid group significantly more negative symptoms ($p<0.01$), no difference in positive, general or total symptom profiles.
Hassiotis (1999)[106]	Subjects with mild learning disability or borderline intellectual impairment[1] determined on basis of NART score.	104	36.5	10.1	482	35	9.0*	Lower IQ group had more negative symptoms ($p = 0.045$)
Bouras (2004)[107]	Subjects comorbid for mild learning disability and schizophrenia spectrum psychosis	53	39.5	matched	53	41.1	matched	LD group showed greater levels of psychopathology on observed, though not reported CPRS. LD higher score on SANS ($p<0.001$)
Chaplin (2006)[108]	Subjects with borderline intellectual impairment[1] determined on basis of NART score.	59	45.4	No data	313	43.2	No data	Borderline intellectual impairment group had higher scores on positive ($p = 0.004$) and negative ($p<0.001$) symptoms scales of PANSS

Table 1.2
Studies investigating the clinical features of schizophrenia in learning disabled populations

Abbreviations: CPRS, Comprehensive Psychopathological Rating Scale; SANS, Scale for the Assessment of Negative Symptoms; PANSS, Positive and Negative Syndrome Scale

[1] 'Borderline intellectual impairment' refers to IQ in range 71-81
[2] Those with 'schizophrenia spectrum psychoses' included rather than just schizophrenia

Discussion

1. Lack of data

As can be seen from the table, there is a paucity of studies investigating the clinical presentation of schizophrenia in learning disability/intellectual impairment. A number of explanations can be postulated to account for this. One reason may be that historically people with learning disabilities were seen as being incapable of suffering from mental illness.[107] This has been strongly refuted by more recent epidemiological studies.[101] Even after this realisation however, studies remained few. This may be a consequence of the fact that the Meadows *et al* study, one of the earliest addressing the clinical presentation of schizophrenia in people with LD, indicated a clinical picture much like that of people without LD.[105]

2. Difficulties in assessing symptom profile in learning disability

It is accepted that the diagnosis of schizophrenia in people with learning disability is hampered by the difficulty of getting clear descriptions of first rank symptoms. Additionally, negative symptoms have the potential to be confused with depression and behaviour which may occur in people with LD who are not mentally ill at all.[109] In mild learning disability however, a number of the widely used structured interviews and rating scales have been shown to have validity,[105,110] and it is generally accepted that in this population symptoms can be characterised with acceptable accuracy. The possibility that negative symptoms are an expression of learning disability rather than schizophrenia can be addressed by including a non-schizophrenic learning disabled control. This was done in the Doody *et al* study, in which it was shown that this group actually scored very low on this symptom subset.[103]

3. Main findings

The most consistent finding from the above studies is that the comorbid group experience a greater weight of negative symptoms. There are a number of possible explanations for this, two being that this group have a longer duration of illness or that they progress more rapidly to a deficit state. In studies that have either matched for illness duration or it is equal, the comorbid group continue to score higher on this symptom subset.[107] This would thus suggest that there is indeed something intrinsic to schizophrenia in the intellectually impaired group which increases the likelihood of reaching a deficit state.

4. Relevance of findings to study proposal

Central to the design of the current study is the idea that co-morbid schizophrenia and learning disability represents a 'severe schizophrenia', in which clinical features, amongst others, are more marked. The finding of more marked negative symptoms in this population is consistent with this concept. Indeed, in a number of these papers it is explicitly discussed that the combination of intellectual disability and schizophrenia could represent a distinct subgroup of people with neurodevelopmental schizophrenia and intellectual impairments.[107,108]

1.9.3 Implications of the concept of a 'severe schizophrenia' for research

The essence of the findings discussed in Section 1.9.1 is essentially that the presence of a schizophrenic diathesis might form the underlying basis of evident

cognitive impairment in some of the young learning disabled population. If this was indeed the case then within the learning disabled population would be some individuals whose learning disability represented a schizophrenic illness in which psychotic symptoms had yet to become manifest. Within the wider population of people with learning disabilities affected individuals are likely to be only a small minority (~3%). Examining the whole population at risk over the period of vulnerability for schizophrenia would not be practical. Findings from the EHRS however suggested a means whereby identification may be facilitated and the issues examined within a pragmatic time frame.

1.9.4 Implications of findings from the EHRS for the identification of learning disabled subjects at elevated risk of developing schizophrenia

The EHRS showed that scores on the Child Behavioural Checklist,[113] (based on parental accounts) and the Structured Inventory for Schizophrenia,[96] (based on subjects' own accounts) were significant predictors of the development of psychotic symptoms and ultimately among the most important predictors of the later development of a formal schizophrenic illness.[97,111,112] As discussed above, though the clinical picture may be more severe in the latter population, there appears to be marked commonality between schizophrenia in the population with normal intelligence and that in the learning disabled population.[104] It may thus be possible, by employing the CBCL and SIS, to identify from within the population with mild learning disability those at elevated risk of developing schizophrenia. By these means a cohort of learning disabled individuals of manageable size can be identified for

study and follow-up, within which a substantial number would be expected to develop the illness

1.10 The amygdala in learning disability

If amygdala volume in learning disabled subjects at high risk for schizophrenia is to be investigated, then it is important to clarify what is known about amygdala volume in non-schizophrenic learning disabled subjects. For this reason a review of studies investigating amygdala volume in idiopathic learning disability was undertaken.

Systematic review of studies investigating amygdala size in learning disability

Medline, EMBASE and PsychINFO were searched for all English language studies published between January 1984 and March 2007 that reported structural MRI data on the amygdala in learning disability. Search terms included 'learning disability', 'intellectual impairment' and 'mental retardation' combined using the AND operator with 'amygdala'. Both free-text and expanded medical subject headings were used. Subject headings were adapted to the specific subject headings of the biomedical databases used. The search strategy was supplemented by including a cited reference search and inspecting the reference lists of included articles. Articles

of interest were those concerning the imaging of individuals with idiopathic learning disability, with or without schizophrenia.

Initial search yielded 128 articles. On review of these abstracts however, only three articles were appropriate for inclusion. Common exclusion criteria were articles addressing defined syndromes associated with learning disability, such as Down's syndrome. The three articles identified were all from the Edinburgh group and discussed the same cohort investigated by Sanderson, described above. Learning disabled controls were included, and as part of a battery of investigations AHC volume determined. One of these studies concerned VBM analysis of the same scans, the other two discussed the same imaging data. Findings from these publications of relevance to this study are detailed below.

Significant findings from Sanderson et al, comparing neuroanatomy in a comorbid learning disability/schizophrenia group with learning disabled, schizophrenic and normal controls by ROI methodology: 1. After controlling for the effects of whole brain volume, left and right AHC significantly smaller in comorbid group than in learning disability group and normal controls. 2. After controlling for whole brain volume, left AHC significantly larger in learning disabled than normal controls 3.Greater right/left difference in AHC size in normal controls

Table 1.3
Significant findings from Sanderson *et al*[104]

Significant findings from Moorhead *et al*, comparing neuroanatomy in a comorbid learning disability/schizophrenia group with learning disabled, schizophrenic and normal controls by VBM methodology. Data was analysed by both normalized and native space analysis; the former controls for whole brain volume, while the latter mirrors ROI analysis uncorrected for whole brain volume.

1. Both the left amygdala and left hippocampus were significantly smaller in comorbids than normal controls by analysis in normalized space, i.e. correcting for whole brain volume.
2. By analysis in normalised space deficits the left amygdala was significantly smaller in the schizophrenic group compared to normal controls.
3. By analysis in normalized space no significant differences were seen between the schizophrenic and comorbid group in volumes of the temporal lobe or amygdalo-hippocampal complex. Overall, this together with other evidence suggested that comorbids and schizophrenics belonged to the same population.
4. Analysis in native space, not correcting for whole brain volume, was also indicative of similarities between the comorbid and schizophrenia groups. This is consistent with the view that comorbid learning disability and schizophrenia is a severe form of schizophrenia.
5. They note that the process of native space analysis could result in a failure to register the expected deficits in schizophrenia and comorbid temporal lobes.

Table 1.4
Summary of findings from Moorhead *et al*[114]

1.11 The Edinburgh Study of Comorbidity

The Edinburgh Study of Comorbidity (ESC) is a large, ongoing prospective study which applies the findings discussed above to the longitudinal assessment of a group of adolescents with special educational needs largely through the presence of a mild or borderline learning disability. As discussed above, by virtue of this cognitive impairment they are already at elevated risk of developing schizophrenia. From this group, by application of the CBCL[113] and SIS[96], those individuals at highest risk of developing the illness should be identifiable. They can then be subject to detailed investigation. Clearly the assumption in employing this methodology is that the two populations (the genetic high risk population in the EHRS, and the cognitively impaired high risk population in the ESC), will prove to show similarities in terms of the pattern of illness development.

The current study

This study concerns a subset of the baseline sample acquired for the ESC. The groups within that sample that I will be discussing are those at highest risk for schizophrenia (learning disabled, CBCL+ and SIS+) and the unrelated, non-learning disabled control group. From the baseline MRI scans of these individuals amygdala volume was determined. This can then be compared between the two groups, and any associations between amygdala size and findings on neuropsychological and psychiatric assessment investigated.

Chapter 2

Methods

2.1 Recruitment

The recruitment process is summarised in Figure 2.1. Recruitment was via schools and colleges across 18 out of the 19 Educational Authorities in Scotland which are within reasonable travelling distance of Edinburgh (one Educational Authority declined to participate). Of the 273 schools and colleges approached 99 agreed to enter the study. The head teachers of these schools were asked to identify adolescents receiving special educational assistance, in particular those with a presumed IQ in the range 50-80. Exclusion criteria at recruitment were known syndromic learning disability, severe cerebral palsy, profound learning disability, lack of speech and a known brain injury.

Parents of the adolescents identified were then invited to participate in the study by letter. Five hundred and one individuals initially agreed to participate although in 36 of these cases, the family subsequently withdrew from the study. The CBCL was, therefore, completed on 465 subjects by one of the research team visiting the home and interviewing the parents. The CBCL has been validated for use in those with learning disability.[115] Of these 465 participants, 42 were excluded and an additional 28 did not participate further (see Figure 2.1). 394 of the 395 were assessed using the Structured Interview for Schizotypy (SIS) (one participant did not complete the SIS). The SIS has not been widely used in the learning disabled population, but a pilot study undertaken by the investigators had confirmed the feasibility of using this tool in the population (there were no subjects in whom the interview could not be conducted and satisfactory inter-rater reliabilities were obtained.[100]

Cut-offs on the scales discussed above, which were found to predict the later development of schizophrenia in the Edinburgh High Risk study,[97] were used to identify those suitable for recruitment into the next phase of the study for more detailed assessment. The average scores on the SIS and the CBCL were higher in this population than in the Edinburgh High Risk Project therefore the cut-off points were scaled up appropriately.[100] Participants were evenly sampled into 4 groups based on these cut-offs – $SIS_{high}CBCL_{high}$, $SIS_{high}CBCL_{low}$, $SIS_{low}CBCL_{high}$ and $SIS_{low}CBCL_{low}$. In addition to the subjects receiving special educational assistance a control group consisting of young people from the same environment was also recruited. The controls had no history of receiving educational assistance or of major psychiatric disorder and were recruited through youth and voluntary organisations.

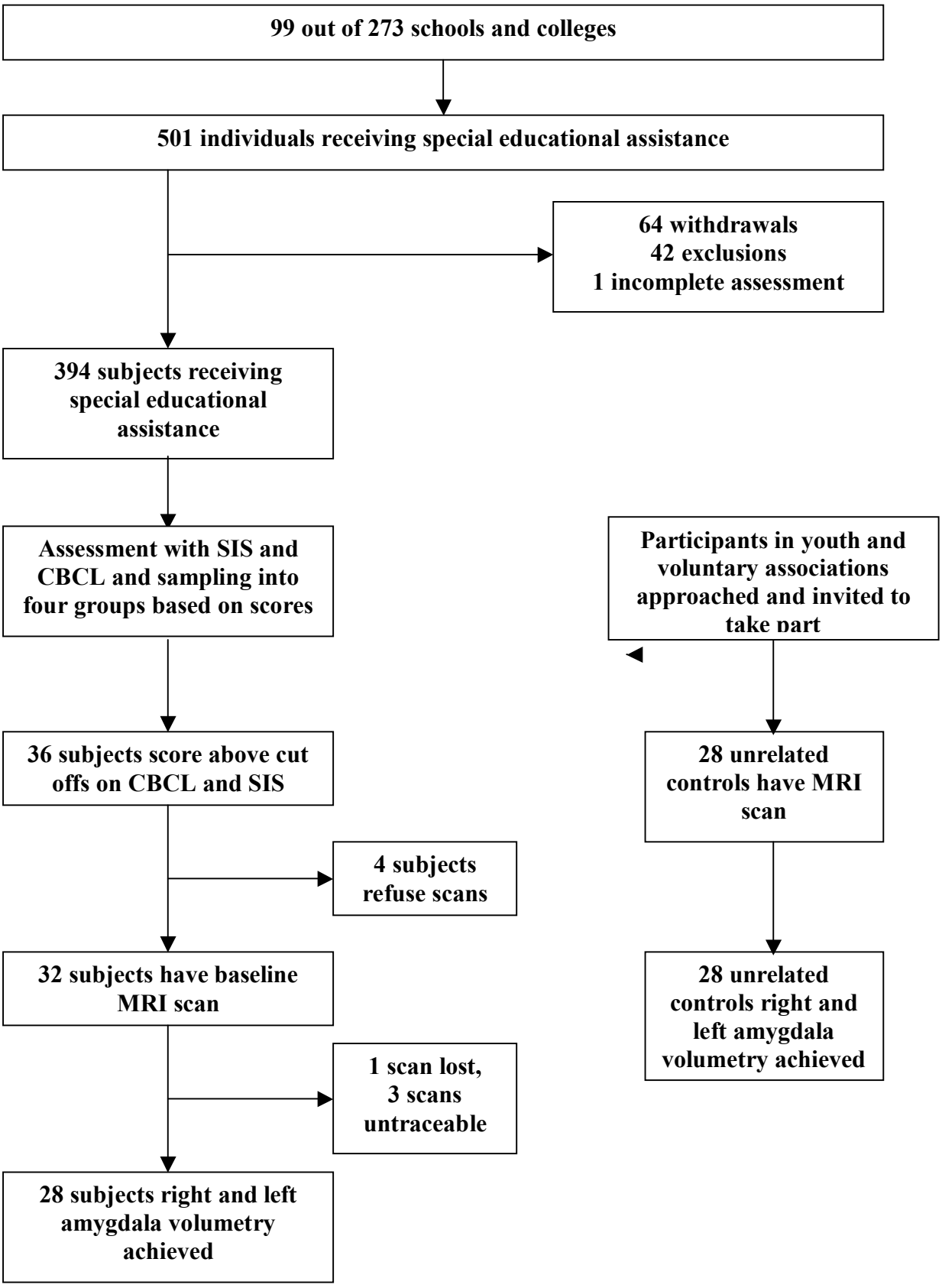

Figure 2.1
Summary of the recruitment process

Following the baseline assessment, generally four to six months later, participants attended for a psychiatric interview and neuropsychological assessment. The neuropsychological testing strategy was tailored to accommodate specific age and ability issues, with an emphasis on memory and executive function. The WISC and WAIS (as appropriate to the individual's age),[116,117] were used to determine IQ in all participants at baseline. Subsequent tests were only undertaken in subjects and not repeated in controls. Amongst these tests was assessment of mental state. The Clinical Interview Schedule (CIS)[118] was chosen for this task rather than the long and complex Present State Examination. It is relatively brief, covers key areas for establishing 'caseness' in major psychiatric disorders with reliability, and is more acceptable to young and particularly to intellectually impaired individuals.[119] Psychotic phenomena were sub-classified as per Goldberg and co-workers in their modification for psychotic subjects.[120] The Positive and Negative Syndrome Scale (PANSS) was also included because of its widespread use.[121] All clinical ratings were done blind to CBCL/SIS cell allocation.

After clinical assessments, subjects had a structural MRI scan. It was from a subset of these scans, those from the CBCL+/SIS+ group and the unrelated control group, that amygdala volume was determined. Of the 36 subjects identified as being in the SIS+/CBCL+ group, 32 were recruited to this part of the study. Of these 32 subjects who were scanned, in the scans of three the amygdalae could not be traced. The reason for this in all three cases was that subjects had been wearing a brace, which obscured the images of the medial temporal lobe structures. One further scan was lost. As can be seen in Figure 2.1, the right and left amygdala of 28 subjects were thus successfully traced. Twenty eight control subjects were also identified and right and left amygdalae successfully traced.

2.2 MRI scanning: Acquisition of scans

MR imaging was performed at the SHEFC Brain Imaging Research Centre for Scotland on a 1.5T GE Signa Echospeed system (GE Medical Systems, Milwaukee, Wisconsin) operating in research mode consisting of a T1-weighted sagittal sequence with parameters of 16/450/0.75 (TE/TR/excitations) and a T2-weighted axial sequence with parameters of 102/6300/2. Volume data was obtained with a 3D inversion-recovery prepared T1-weighted sequence with parameters of 3.3/8.1/1, TI 600, flip 15, slice thickness 1.7 mm (no gap), matrix 256×192, FOV 220 mm.

2.3 Method of amygdala measurement

Background

In vivo assessment of amygdala volume by magnetic resonance imaging is generally recognised as challenging. Unfortunately, the amygdala and structures surrounding it have similar signal intensities, a fact which makes accurate delineation of amygdala boundaries difficult. Perhaps unsurprisingly therefore, a striking feature of studies that have addressed the measurement of the amygdale in subjects with schizophrenia is the wide range of volumes encountered. This is equally true of studies in normal subjects, with reports of volumes ranging from 1 to 4 cm^3.[122] Another striking finding is the variety of protocols that have been devised with the

aim of achieving reliability in amygdala measurement, and the variety of different landmarks utilised in these. Despite these obvious difficulties, for this study to have validity it is essential that an accurate method for identification and delineation of the amygdala can be employed.

Brierley *et al* published a systematic review and meta-analysis of amygdala volumetric magnetic resonance imaging in 2002.[123] Their aim was to estimate the normal range of amygdala volume in adults and to account for the heterogeneity of previously reported measures. Among other factors, they considered subject and methodological variables, positional and volume correction and the reliability of measurement. They discuss the tendency, given that amygdala borders are not clear, for methodologies to tend to rely on external landmarks to improve reliability. Clearly this means that individual anatomical variation can influence measures of amygdala size.

The Brierley study used a wide variety of studies to provide data on volumetric MRI of the adult human amygdala in non-clinical subjects. Studies were only included if there was adequate description of the methodology employed in the anatomical definition of the amygdala or a suitable reference cited and volume measures were not given in combination with the hippocampus. These studies utilised various measurement protocols, and thus enabled comparison of anatomical definitions of the amygdala.

Brierley *et al*'s review included 39 publications. They found that the main methodological factor found to influence amygdala measurement was anatomical definition. Of the various methodologies employed to delineate the amygdala in these studies, Watson's[124] was employed in a third. When 95% confidence intervals for both right and left amygdala volumes obtained by these criteria were compared to

those obtained by 'non-Watson' criteria, there was no overlap. The former method yielded measurements of 2384-2539.6mm³ and 2219.3-2373.7 mm³ for left and right amygdala respectively, while with the latter methods these figures were 1612.2-1690.6 mm³ and 1582.4-1667.0 mm³.

As discussed above, it is clear that significantly greater volumes are generated by employing Watson's criteria. Given the heterogeneity of the 'non-Watson' group, it is difficult to compare the sets, but what is apparent on studying Watson's criteria is that they rely heavily on external landmarks. It thus seems evident that that means of determination of boundary definition will have an impact on amygdala size, and it is conceivable that heavier reliance on external landmarks could skew amygdala volume estimation. It must be remembered that the individuals included in this study were non-clinical subjects. If we are to accept the suggestion of Breierley *et al*, that for reasons of consistency Watson's criteria should be employed, then we are also assuming that this practice is equally applicable to clinical subjects.

The issue of reliance on external landmarks in amygdala measurement, and its influence on amygdala size estimation in clinical subjects is further discussed in the paper of Chance *et al*, also of 2002.[79] They reflect on the potential pitfalls of employing this technique when schizophrenia is known to be associated with significant anatomical variations from normal. For example, it is reported that temporal lobes are shortened in this condition; if this is the case, then landmarks that lie outside the temporal lobe will be further forwards relative to structures within the temporal lobe. Consequently, the use of external landmarks in MRI studies could constitute a source of systematic error, yielding smaller amygdala size in these subjects.

Review of methodologies employed in delineation of the amygdala from 2004-2007

From the discussion above it seems clear that, in subjects who are not 'normal' at least, there is an argument against the widespread adoption of Watson's criteria. For these subjects methods of volume estimation from MRI images that are less dependent on external landmarks would seem to have advantages. To determine what has been general practice in recent years (when it should be more viable to be less dependant on external landmarks, given greater scan resolution), I undertook a review of methods of amygdala measurement that have been employed within the three year period from 1st January 2004 to 1st January 2007. As Brierley *et al*'s meta-analysis was published in 2002, the bulk of studies published within this time period would likely have commenced after this publication was available. The first aim of this review was to establish if consensus on anatomical delineation of the amygdala has occurred. If this is the case then it would be reasonable to employ this methodology in this study. If this consensus has not occurred, then this review will also assist in identification of an appropriate methodology to utilise in this study. This would ideally have both high reliability and minimal reliance on external landmarks.

Method

In the case of Brierley et al's extensive review, their computerised literature search using Medline and dating up to February 2001 was performed using 'amygdala', combined with 'magnetic resonance imaging' as search terms. On running a search with the same parameters on PubMed for the three year period 1st January 2004 to 1st January 2007, 507 articles were identified, of which 59 were

review articles. Review of this number of abstracts was beyond the scope of this work, and so a further search was run to try and focus on those articles more likely to have directly addressed the issues involved in measurement of the amygdale. For this purpose a search was run using the key word 'amygdala' combined with the AND operator with 'magnetic resonance imaging' AND 'measurement' or 'volume' or 'protocol'. This identified 178 articles, of which 14 were reviews. Inclusion criteria were that the study was printed in English and employed structural MRI and region of interest methodology to determine volume of the amygdala. On review of these abstracts 42 papers seemed appropriate for inclusion, but only 31 were obtainable in full text form. As the main role of this review was simply to gain an impression of the range of techniques employed in amygdala delineation in this time period, this seemed a reasonable yield. The papers were reviewed to determine the technique employed in amygdala delineation. If this was not clear, then this is stated in the summary of findings below.

Study	Subject group	Method of amygdala delineation	Reliability	
			Inter	Intra
Szeszko, PR *et al* 2004[125]	Paediatric cases with OCD	Watson		0.93 (L) 0.85 (R)
DelBello, MP *et al* 2004[126]	Adolescent biploar	Altshuler method-based on external landmarks	>0.9	>0.9
Reiss, AL *et al* 2004[127]	Williams syndrome	Used 'detailed on-line protocol' but no link given	>0.9	
Shumann, CM *et al* 2004[128]	Children and adolescents with autism	Described own method, relatively low use external landmarks	0.92(L) 0.93(R)	>0.95
Brombilla, P *et al* 2004[129]	Borderline personality disorder	Method previously described by Brombilla *et al.* Heavy use of external landmarks	Not recorded	
Lindauer, RJ *et al* 2004[130]	Dutch police officers with PTSD	Bogerts, with modification	0.95(L) 0.95(R)	
Rojas, DC *et al* 2004[131]	Parents of children with autistic disorder	Honeycutt	0.95	0.97
Makris, N *et al* 2004[132]	Cocaine addicts	Largely based on method previously described by Makris. Minimal use external landmarks.		0.84
Niu, L *et al* 2004[133]	Patients with schizophrenia	Convit	0.98	0.99
Rosso, IM *et al* 2004[134]	Paediatric major depression	Shenton		>0.90
Adolphs, R *et al* 2005[135]	People with damage to medial temporal lobe	'traced by two technicians'	>0.90	
Kalus, P *et al* 2005[136]	Schizophrenia	Pruessner		0.93(L) 0.94®
Tanskanen, P *et al* 2005[137]	Schizophrenia and other psychoses	Lehernicy	0.87(L) 0.86(R)	
Basso, M *et al* 2006[138]	Alzheimer's disease	Watson, with some modifications	0.85(L) 0.80(R)	

Yoshikawa, E *et al* 2006[139]	Cancer survivors with intrusive recollections	Combination of Pruessner, Watson and Convit	0.82 0.94
Chey, J *et al* 2006[140]	Non-demented elderly individuals with poor cognitive function	Watson	0.92
Horineck, D *et al* 2006[141]	Alzheimer's disease	Delineation 'according to multiple sources, including histological sections and neuroanatomic atlases'	0.94
Zetzsche, T *et al* 2006[142]	Borderline personality disorder and depression	Convit, with slight modifications	0.93 0.91
Velakoulis, D *et al* 2006[143]	Schizophrenia and high risk for schizophrenia	Convit, with slight modifications	0.79(L) 0.88(L) 0.70(R) 0.87(R)
den Heiger, T *et al* 2006[144]	Cognitively intact elderly people	'with reference to an atlas'	0.77(L) 0.82(L) 0.80(R) 0.78(R)
Blumberg, HP *et al* 2006[145]	Adolescents/young adults with bipolar affective disorder	Combination of Kates and Schumann	0.96(L) 0.91(R)
Barkataki, I *et al* 2006[146]	Men with schizophrenia or antisocial personality disorder	'posterior by H-A transitional area, anterior by fronto-temporal junction, laterally by surrounding parahippocampal white matter and CSF'	0.95
Teipel, SJ *et al* 2006[147]	Mild Alzheimer's disease	Preussner	>0.90
Goncalves Pereira, PM *et al* 2006[148]	Pharmaco-resistant TLE	Watson	'intra-observer variability 3.3%
Dziobek, I *et al* 2006[149]	Adults with Asperger syndrome	Convit	0.93
Vermetten, E *et al* 2006[150]	Dissociative identity disorder	Watson	0.98
Kates, WR *et al* 2006[151]	Children with velocardiofacial syndrome	Kates, low reliance external landmarks	0.95

Munson, J *et al* 2006[152]	Children with autism	Honeycutt	0.90 0.97
Debbane, M *et al* 2006[153]	22q11.2 deletion syndrome	Kates	0.93

Table 2.1
Studies in which the amygdala was measured by Region of Interest methodology in the period 2004-2007

Discussion of range of methodologies employed

On review of the data above it is clear that, despite Brierley's paper, consensus regarding the technique that should be employed in delineation of the amygdala has certainly not been reached. It is the case that of all methodologies Watson's is the most prominent; it was the single most used technique, being employed in 6 of the 31 studies. The methodology described by Convit[154] was second most commonly used, being chosen in 4 studies. In a total of five studies the methodology employed was far from clear.

As there is clearly no consensus on techniques employed, it is important to consider the merits of the various methodologies. As outlined by Chance *et al*, and discussed above, the extent to which a methodology relies on external landmarks influences its susceptibility to the introduction of systematic bias.[79] Of the techniques discussed, Convit *et al*'s does try and minimise use of external landmarks, though they are employed in determination of the anterior extent. Pruessner's technique is more dependant on external landmarks, particularly for the superomedial amygdala.[122] The landmarks employed in Schumann's technique abut directly on to the amygdala.[128] This should mean that the influence of anatomical abnormalities in calculation of amygdala volume are minimised. This technique also managed to achieve satisfactory reliability.

Implications of review for methodology employed in this study

In the present study the aim was to compare amygdala size in a group with learning disability and various other markers of elevated risk for schizophrenia with normal controls. The former group is clearly likely to have abnormalities of brain structure. It does thus seem that, at least if satisfactory reliability could be achieved, a method of delineation of amygdala boundaries which did not rely on external markers would be optimal. This would prevent abnormalities of structures surrounding the amygdala skewing amygdala measurements. On this basis the protocol for amygdala measurement as outlined in the Appendix was devised. Though there are some modifications, it relies heavily on the methodology outlined by Schumann.[128] Reliability (intraclass correlation coefficients) of measurements using this protocol was then established. Either right or left amygdalae from seven brains (selected at random) were traced twice to establish intra-rater reliability. Ten amygdalae (again selected at random) were also traced by a second investigator (AS) to establish inter-rater reliability. Inter-rater reliability was 0.80 and intra-rater reliability 0.88. I regarded this as satisfactory.

2.4 Statistical analysis

All statistical analyses were carried out using SPSS 14.0 for Windows. Differences between the demographic characteristics were assessed using t tests and chi sq as appropriate. The amygdala volume data were evaluated to determine the

appropriateness of using parametric statistics. The distribution of the data was approximately normal, and the ratio of standard deviation to mean compatible with normalcy in all groups. The appropriateness of employing parametric statistics is discussed in more detail in Chapter 4. Mean amygdala volume in the two groups was compared using the independent t-test, both with and without modification for unequal variances. Given that this inequality of variance did exist between the two groups, (that in the unrelated controls was just over double that in the subjects), I also undertook this comparison using non-parametric statistical methods. Thus, I also compared amygdala volume in the two groups with the Mann-Whitney U-test. This enabled comparison of the results both with and without the assumption of equal variance and with and without the assumption of normalcy. As will be seen in Section 3.1, the results of all three analyses were similar. Separate analyses of covariance (ANCOVA) were used for each hemisphere to examine for differences in amygdala size between the groups. Amygdala size was the dependent variable with group and gender as fixed factors. The analysis was repeated with the addition of whole brain volume as a covariate. Standardised residuals were checked for normality.

Analysis of within study group differences was also undertaken. The associations between amygdala size and scores on symptom scales and the relationships between age and amygdala volumes within the study group were examined using partial correlations covarying for whole brain volume and gender. The relationships between age and symptom ratings in each group were assessed using Pearson's r.

Chapter 3

Results

3.1 Comparison of study and control groups

3.1.1 Descriptive data

The basic characteristics of gender, age at first assessment, height, IQ, scores on the CBCL and SIS and whole brain volume of the two groups under study are detailed in Table 3.1. A summary of statistical comparison is also included. As expected, given that the group was selected on the basis of scores on these tests, the CBCL+/SIS+ group has significantly higher mean scores on the CBCL and SIS than the control group. Also unsurprisingly, the unrelated control group had significantly higher mean IQ. Comparison between groups of height, age at first assessment and whole brain volume revealed no significant difference. Comparison of gender did however, with males being over-represented in the study group. This reflects the substantial difficulties in recruiting male control subjects for the study.

	CBCL+/SIS+	**Controls**	**P value**
Age at first assessment	16.1 (1.9)	16.6 (1.7)	0.32
Number of subjects	28	28	
Height	166.9 (9.6)	169.5 (8.8)	0.30
Full IQ	74.6 (18.0)	107.4 (15.3)	0.00
Gender (M:F)	20:8	10:17	0.01*
SIS	38.5 (8.0)	19.3 (5.4)	0.00
CBCL	111.8 (17.9)	13.9 (12.4)	0.00
Whole brain volume	1352745 (176082)	1359057 (147109)	0.89

Table 3.1
Demographic characteristics, IQ and mean CBCL and SIS scores for subjects and controls.
Aside from for gender results are shown as mean (standard deviation).
Statistic is independent samples t test aside from * which is chi squared.

3.1.2 Comparison of amygdala volumes

Descriptive statistics of amygdala volumes are summarised in table 3.2. Data for right and left amygdale are displayed separately as there is substantial data suggesting that there is asymmetry of amygdala size.[44,51] Mean amygdala volume for males and females has been combined.

	Left amygdala		**Right amygdala**	
	CBCL+/SIS+	Controls	CBCL+/SIS+	Controls
Number of subjects	28	28	28	28
Mean	1405.3	1282.9	1436.4	1288.3
Standard deviation	334.9	158.7	386.5	135.5
Median	1498.8	1254.2	1435.0	1291.3
Range	1394.9	576.3	1843.1	572.5

Table 3.2.
Raw amygdala volume (mm^3) in CBCL+/SIS+ and control subjects.

As can be seen from table 3.2, mean amygdala volume is greater in the study than control group. The significance of this difference in the raw data was investigated using both parametric (independent t-test) and non-parametric (Mann-Whitney U-test. The independent t-test was employed both with and without modification for unequal variance. The rationale behind using both tests is discussed in Section 2.4, and results shown in tables 3.3 and 3.4.

As can be seen in Table 3.3, the difference in volume between the study and control groups was close to significance. This was particularly the case for the right amygdala, the level of significance seen being similar both when parametric and non-parametric statistics were employed.

	Left amygdala			Right amygdala		
	Difference in means	95% confidence interval of difference	Sign.	Difference in means	Confidence interval	Sign.
Equal variances assumed	122.4	-18.0 to 262.8	0.086	148.1	-7.2 to 303.4	0.61
Equal variances not assumed	122.4	-19.3 to 264.0	0.089	148.1	-9.4 to 305.6	0.65

Table 3.3
Comparison of raw amygdala volumes in SIS+/CBCL+ and control subjects by the independent t-test both with and without the assumption of equal variances.

	Mean rank		Sum of ranks		Z score	Sign.
	CBCL+ SIS+	Controls	CBCL+ SIS+	Controls		
Left amygdala	32.04	24.96	897.00	699.00	-1.62	0.105
Right amygdala	32.50	24.50	910.00	686.00	-1.84	0.066

Table 3.4
Comparison of raw amygdala volumes in SIS+/CBCL+ and control subjects by the Mann-Whitney U-test.

3.1.3 Comparison of amygdala volume with adjustment for potential confounders

As can be seen in Table 3.1, there is a gender imbalance between the study and control groups and they also differ (albeit to a lesser extent) in whole brain volume. To enable these differences to be taken into account in the analysis, analysis of covariance was undertaken. Amygdala volume was the dependent factor, group and gender fixed factors, and whole brain volume a covariate. Analysis was repeated with full IQ as an additional covariate. Results are shown in Tables 3.5 and 3.6.

	Mean amygdala volume		F statistic	Significance
	SIS+/CBCL+	Controls		
Left amygdala	1400.9	1280.1	2.949	0.092
Right amygdala	1402.7	1277.2	2.425	0.126

Table 3.5
Comparison of amygdala volume in study and control groups by ANCOVA with covariation for gender and whole brain volume.

	Mean amygdala volume		F statistic	Significance
	SIS+/CBCL+	Controls		
Left amygdala	1429.7	1254.5	3.615	0.063
Right amygdala	1466.2	1200.9	5.663	0.021

Table 3.6
Comparison of amygdala volume in study and control groups by ANCOVA with covariation for gender, whole brain volume and full IQ.

As can be seen in Table 3.6, when IQ is included as a covariate the volume of the right amygdala is larger in the study group compared to the controls to a statistically significant degree. Overall however covariation for IQ was no felt to be appropriate in the context of this study; the reasons for this are discussed further in Chapter 4.

3.2 Relationship between amygdale volume of study subjects and other baseline measures.

3.2.1 Relationship between amygdala volume and score on PANSS

Correlation between amygdala volume, (after covariation for gender and whole brain volume), with PANSS score is shown in Table 3.7. As well as total PANSS score, scores on the positive symptom, negative symptom and general symptom components of the rating scale were assessed.

		PANSS positive	PANSS negative	PANSS general	PANSS total
Left amygdale	Correlation	-0.048	-0.511	0.062	-0.308
	Significance	0.821	0.009	0.767	0.134
Right amygdale	Correlation	-0.051	-0.261	0.071	-0.110
	Significance	0.810	0.208	0.735	0.600

Table 3.7
Correlation between score on PANSS and amygdala size within the study group after covariation for whole brain volume and gender

A significant correlation was seen between score on the negative subset of symptoms on PANSS and left amygdala size covaried for gender and whole brain volume. This correlation is shown graphically in Figure 3.1. This correlation remained significant after the addition of age as a covariate.

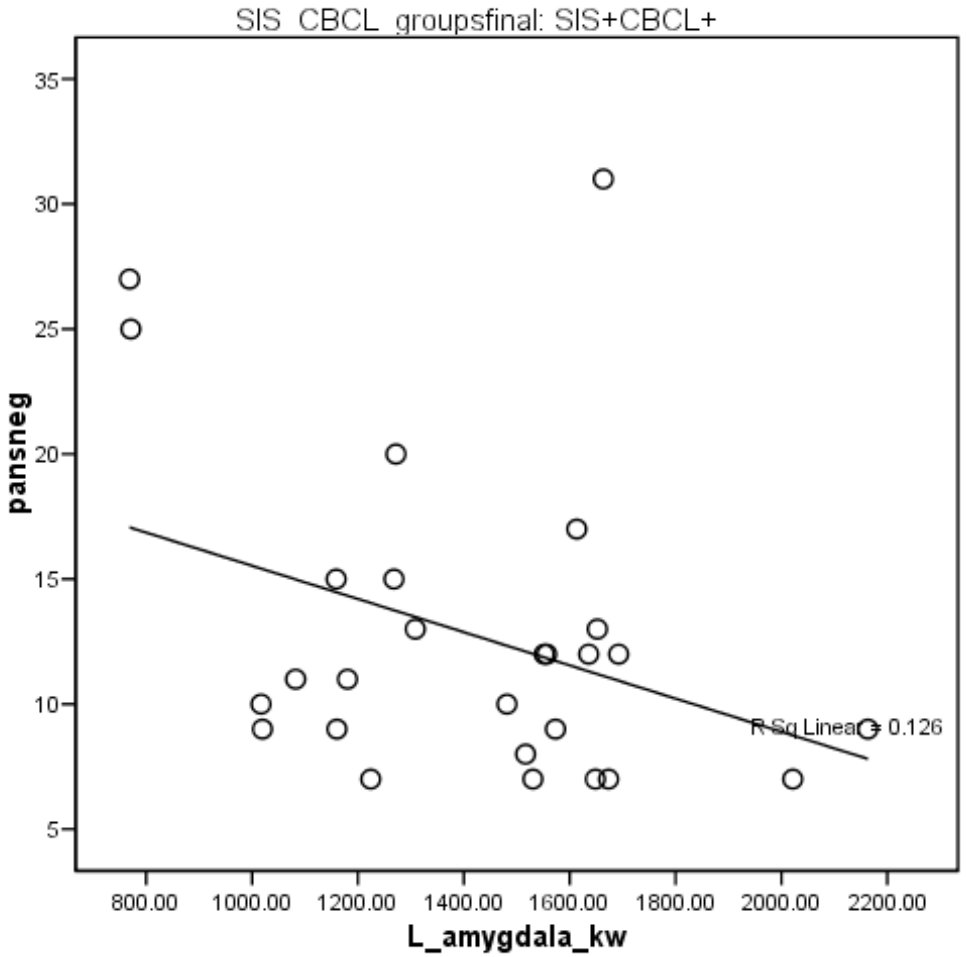

Figure 3.1
Relationship between score on PANSS negative symptom subset and left amygdala volume with covariation for gender and whole brain volume.

3.2.2 Relationship between amygdala volume and score on CBCL

Correlation between amygdala volume, (after covariation for gender and whole brain volume), and score on the CBCL is shown for the SIS+/CBCL+ group in Table 3.8. As can be seen the correlation was not significant. This remained the case after the addition of age as a covariate.

	Correlation	Significance
Left amygdala	0.199	0.329
Right amygdala	0.062	0.764

Table 3.8
Correlation between score on CBCL and amygdala volume within the study group after covariation for gender and whole brain volume.

3.2.3 Relationship between age and score on symptom scales.

The relationship between age and scores on symptoms subgroups as measured by PANSS was also examined for the study group and the results are shown in Table 3.9. As can be seen a significant relationship was seen between age and score on the PANSS positive and general subsets, and a highly significant relationship between age and score on the PANSS total and PANSS negative subsets. The relationship between score on the negative symptom subset of PANSS and age is shown in Figure 3.2.

	PANSS positive	PANSS negative	PANSS general	PANSS total
Pearson's correlation	0.427	0.638	0.473	0.658
Significance	0.026	<0.001	0.013	<0.001

Table 3.9
Correlation between PANSS scores and age for study group.

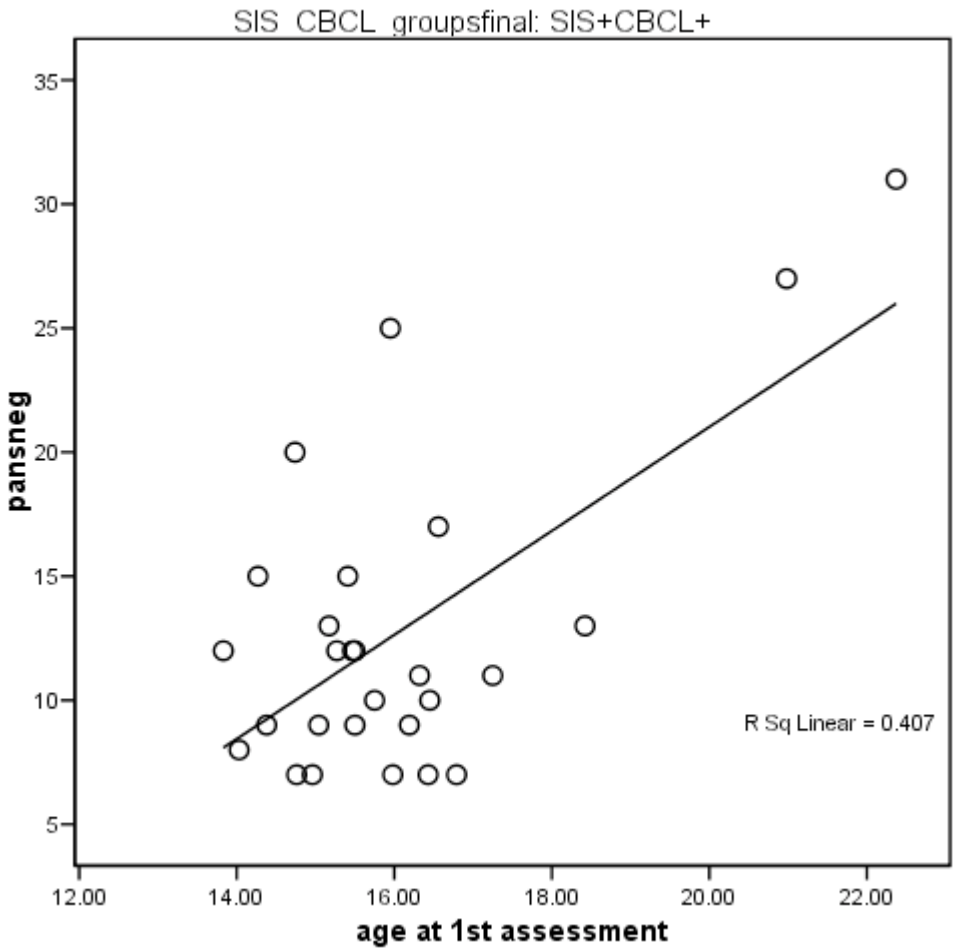

Figure 3.2
Correlation between score on negative subscale of PANSS and age at first assessment.

3.3 Comparison of relationship between volume of left and right amygdala and age in the study and control groups

As is discussed above, there is a significant association between increasing age and greater weight of negative symptoms. There is also a significant association between greater weight of negative symptoms and reduced left amygdala volume within the study group. This naturally leads to speculation about the relationship

between age and amygdala volume. Investigation of this relationship is detailed in Table 3.10.

		Correlation	Significance
CBCL+/SIS+	Left amygdala	-0.325	0.106
	Right amygdala	-0.175	0.393
Controls	Left amygdale	-0.120	0.560
	Right amygdala	0.134	0.513

Table 3.10
Correlation between amygdala volume and age in the study and control groups. Gender and whole brain volume have been controlled for.

As can be seen in Table 3.10, there is no significant relationship between age and amygdala volume in either the study or control groups. There is a tendency for a greater negative relationship between left amygdala volume and age in the study group than the control group although this difference was not significant.

Chapter 4

Discussion of statistics

Use of parametric statistics

The use of parametric statistics is dependent on a number of assumptions being made about population distributions and parameters. Namely, the populations are assumed to have normal distributions and equal variances. Justification on the former count was provided by checking the standardised residual plots for normality and finding them satisfactory. The requirement for comparable variance was more problematic. When checked by Levene's test, this was a significant difference, and it is acknowledged that this can potentially result in a type 1 error.[155] It is accepted however that when sample sizes are equal this does mitigate the effect of unequal variances and in these circumstances the use of parametric statistics can be justified.[155] Given this, together with the fact that group sizes were reasonable, I felt that parametric statistics could be applied to this data set. As there are theoretical concerns about criteria being satisfied however I also compared the means of the two groups using non-parametric methods. The results employing parametric and non-parametric methods were comparable.

Covariation for IQ

On comparison of CBCL+/SIS+ and control raw data, it can be seen that amygdala size is greater in the latter, for both left and right amygdala in males and females. When data for each sex are combined and gender covaried for this trend persists, though does not reach statistical significance. When data is covaried for IQ as well as gender however, for the right amygdale at least, the difference is statistically significant. On analysis, a significant group by IQ interaction is seen. This inevitably brings into question the appropriateness of covarying for IQ.

In the design of the ESC, both normal and learning disabled controls were included. Ideally of course, amydala volume in the study group would be compared to both controls. Unfortunately however, determination of amygdala volume in the learning disabled control group was outwith the scope of this study. Covariation of the data for IQ could potentially allow more appropriate comparison of these clearly very different groups. Covariance is often employed in matching designs to control for factors that have not been matched for.[28] One disadvantage of this approach however is that it assumes certain statistical properties of the data (normalcy, variation); this issue is discussed above. Additionally, the result of analysis of covariance are considerably less interpretable if the groups differ substantially on the covariate in question,[28] a fact clearly relevant to this data. It also requires that all subgroups within the data set show the same pattern of relationship between amygdala size and IQ, a fact that may be particularly questionable within the heterogeneous study group. Perhaps the strongest argument against covariation for IQ however comes from the premise for the study itself. The rationale for the study rests on the assumption that low IQ may be a manifestation of undiagnosed schizophrenia. Thus, if IQ is controlled for it is possible that structural abnormalities associated with this prodromal state could be artificially obscured.

Covariation for whole brain volume

It is recognised that variation in the sizes of specific brain structures tends to be correlated with normal variation in brain size. Thus, people with larger brains tend to have larger brain structures, lateral ventricles, grey matter, and so on. A positive relationship has specifically been reported between head size and amygdala

volume.[156] Thus, brain size can potentially introduce confounding variance or 'noise' into quantitative brain data. Accordingly, many researchers have tried to reduce the influence of brain size variation, producing structural data corrected for brain size. Various methods can be employed to do this. The two most commonly used are either creation of a structure of interest to brain volume proportion or multivariate techniques such as analysis of covariance to covary data for whole brain volume. It has been argued that the latter is more appropriate.[157,158]

Despite the above, it has also been argued that data should be presented in the most direct way, with raw volumes used. Bogerts *et al* discuss that though sex differences do need to be controlled for, use of raw volumes is valid for within group comparisons.[159] Though clearly a heterogeneous group, the analysis of association between score on PANSS and amygdala size within the high risk group could be regarded as such a situation.

It is certainly not universal practice to adjust data to take account of whole brain volume, and given the above arguments I have presented data in the raw form. On considering these arguments however, and particularly given that the study group is known to be heterogeneous, it seems most appropriate for analyses to take account of whole brain volume. As it is generally believed that the most appropriate adjustment is analysis of covariance, this is the method I have employed.

Multiple comparisons

It needs also to be acknowledged that much data in this study has been subject to multiple comparisons. This does of course bring with it the risk of a type 1 error.

Though it is the case that the hypotheses being tested were planned, rather than post hoc, this fact must still be acknowledged. As such, findings are best regarded as hypotheses generating rather than hypotheses confirming.

Chapter 5

General Discussion

5.1 Part 1 – Introduction to discussion

Any comparison of the amygdala volumes observed in the SIS+/CBCL+ and control groups must be placed within the context of normal amygdala development during adolescence. This is particularly important as it has been proposed that adolescence is a period associated with substantial neurobiological change, and more specifically that it involves a shift from greater limbic to prefrontal cortical control of behaviour.[160] These neural changes are believed to underlie a shift from behaviour that is driven by affective impulses to more regulated behaviour that is guided by consideration of future personal and social consequences.[161] As discussed in Section 1.1, there is evidence to suggest that the amygdala has a role in emotional recognition and experience, motivated behaviour and social cognition; these are spheres that clearly have potential import in the processes undergoing development during adolescence. Given these postulated roles, it is thus unsurprising that the amygdala has been suggested to be one of the brain regions to undergo change in the process of adolescent brain development.[162]

In light of the above it is surprising that there are in fact very few studies employing neuroimaging to investigate amygdala development during adolescence. I was able to identify only one longitudinal study providing such data, the control group of the NIMH study of childhood onset schizophrenia.[73] Thirty-six subjects from the control group had longitudinal scans. They had a mean age of 13.6 (SD 2.6) at the time of the initial scan, and 16.2 (3.1) at the time of the second scan. Statistical analysis employed methodology that combined cross-sectional and longitudinal data. From this analysis they concluded that in normally developing individuals amygdala volume does not change significantly within the age range investigated (13-18).[73]

Though the NIMH study is the only I could identify incorporating longitudinal data, others have directly addressed the using cross-sectional techniques. The largest of these, from the same group as the study above, involved 99 children aged 4-18 years. The left amygdala was found to increase significantly with age, but only in males.[163] Schumann *et al*'s study of the amygdala in autism included 27 typically developing (all male) controls.[128] In this case the controls were divided into two groups of subjects (one aged 7.5-12.5 years, the other 12.5-18.5 years); the volume of the amygdala in the older group was significantly greater than in the younger group. This was despite the older group having significantly smaller total cerebral volume. In contrast to these two findings are those from a cross-sectional study by Chen *et al* investigating amygdala development in adolescents with bipolar disorder and including 21 healthy controls (9 female and 12 male; age 11-21).[164] In this study a significant inverse relationship was seen between age and volume of the left amygdala in males (and they alone). It is the case that many of the subjects in the latter study will be post adolescence, but the range of ages included is comparable to the present study.

In summary, though there is a paucity of longitudinal studies, what evidence there is does suggest that, (for males at least), the process of transition from early to late childhood may involve an increase in amygdala volume. In the period of later adolescence/early adulthood it is unlikely there is any increase in volume, and (for males at least), there may actually be a decrease. It is this age range which is the focus of the present study.

An additional important additional consideration when investigating subtle differences in small, subcortical structures is that there is an inherent limit to the resolution of the imaging technique employed. This further hampers the accurate

delineation of anatomical boundaries,[79] and makes it particularly difficult to identify any structural changes that may be occurring when, for example, an individual undergoes the process of transition to schizophrenia. In short, even if differences from controls are not detected, it does not mean they are not present.

5.2 Part 2 – Comparison of amygdala volume in the study and control groups

Amygdala volume was not reduced in the SIS+/CBCL+ group

While acknowledging the limits inherent to the techniques employed, it had been expected that amygdala volume in the CBCL+/SIS+ group would be reduced compared to controls. This expectation is based on four central findings, discussed in the introduction: 1) Amygdala volume is reduced in those with schizophrenia, 2) The volume of the AHC is reduced in those at high risk of schizophrenia, 3) Individuals comorbid for learning disability and schizophrenia have reduced volume of the AHC, 4) Within a high risk population those who will develop schizophrenia can be identified by scores above cut offs on the CBCL and SIS. This was not seen however. Indeed, after covariation for gender and IQ (the latter being a questionable practice, as discussed above), right amygdala volume was significantly greater in the study group than controls. Even without covariation for IQ there is a definite bilateral trend towards larger amygdalae in the study group which approached significance. There are a number of possible explanations for this unexpected finding, and these are outlined below.

5.2.1 It is incorrect to expect reduced amygdala size in an adolescent population at elevated risk for schizophrenia.

Though there is substantial data that amygdala volume is reduced in established schizophrenia, the bulk of this data originates in adult populations. Data concerning amygdala volume in younger populations, as discussed in Section 1.3, is more inconsistent on this matter. The EHRS did of course involve an adolescent group but, as will be discussed later, in this study the amygdala and hippocampus were measured together. One particular data set of note is that of Levitt *et al*, who in their study of children with schizophrenia (mean age 14.2, SD 3.8 years) found that amygdala volume was significantly larger in subjects with schizophrenia than controls.[70] There are clearly major differences between the cohort in the current study and that in Levitt *et al*; not least they are older (mean age16.1, SD 1.9), and do not have schizophrenia. Nonetheless, this finding would clearly fit with the finding of larger amygdalae in a population thought to be at elevated risk of schizophrenia. Levitt *et al* suggest a hypothesis to explain the larger amygdalae of initial sparing of medial temporal regions in childhood-onset schizophrenia, which may then be overcome by a degenerative process associated with illness progression.[70] This may of course have relevance to the cohort investigated in this study.

5.2.2 Heterogeneity of study group

It is possible that although there is indeed a population within the CBCL+/SIS+ group that is destined to develop schizophrenia, other populations are

also present. One of these populations may be a 'normal' learning disabled group. It was shown by Sanderson *et al* that the 'normal' learning disabled population has a larger left amygdalahippocampal complex than controls.[104] Admittedly this was only after controlling for whole brain volume, raw mean amygdala volume being slightly smaller. Even in the case of raw mean volumes however, the standard deviation in the learning disabled group was greater than controls, indicating a greater range of volumes. Though findings are contradictory, some studies have also found an enlarged amygdala in subjects with autism.[128] This is of relevance to this study, as some subjects with autism may have been selected into the study group by the use of the CBCL and SIS. This possibility is discussed in more detail in Section 5.6. If it is the case that autistic and/or 'normal' learning disabled subjects have been included in the study group, we would thus expect to see a wide range of amygdala volumes, individuals being present with amygdala both smaller and larger than those seen in the control group. This is indeed the pattern seen.

An alternative cause of heterogeneity of amygdale size is also suggested by the work of Sanderson.[165] In her study investigating correlations between historical variables and cerebral structure in subjects comorbid for learning disability and schizophrenia, she noted a significantly larger right AHC in those who had experienced obstetric complications compared to those who had not. Again this was relative to whole brain volume. It is also conceivable that within the CBCL+/SIS+ group there are subjects whose clinical presentation is caused by a distinct aetiology such as this and has different structural associations.

5.2.3 The CBCL and SIS do not predict those at elevated risk for schizophrenia in a learning disabled population

Scores on the CBCL and SIS were shown to predict those who developed schizophrenia in a population at high genetic risk for the condition.[97] It is conceivable this is not the case in a learning disabled population. Thus, it may be that for these individuals their learning disability is not a prodromal state, and they are not destined to develop schizophrenia. If this population do indeed represent a 'normal' variant of learning disability, then as was shown for the 'normal' learning disabled population,[104] larger amygdala volume would be expected. The fact is however, that for the SIS in particular, the nature of the symptoms bear such similarities to schizophrenia, that it seem unlikely a subgroup of the learning disabled population scoring highly on this measure could be regarded as a normal population. Even if this group is not destined to develop schizophrenia, they clearly score highly on schizotypal symptoms; as reviewed in Section 1.8, though inconsistent, there are reports that schizotypy per se is associated with reduced amygdale volume.[40]

The argument that the CBCL and SIS do not predict schizophrenia in a cognitively impaired population is further undermined by recent findings from the ESC.[100] During the time scale of the study three cognitively impaired individuals from the high SIS group have gone on to develop schizophrenia, and a further six have symptoms highly suggestive of the condition. This is substantially more than would be expected by chance alone, and it does thus seem that these relatively simple tools do indeed predict increased vulnerability to schizophrenia within this population.

5.2.4 It is volume differences in the hippocampus, not amygdala that were detected in the study of subjects comorbid for learning disability and schizophrenia .

As discussed in Section 1.9, AHC volume is reduced in those at high risk for schizophrenia, and in those comorbid for learning disability and schizophrenia. Given the limitations of imaging techniques at the times of these studies, the amygdala and hippocampus could not be accurately separated, and so were measured together. In this study the amygdala could be delineated and so was measured separately. It is of course possible that AHC volume is indeed reduced in the CBCL+/SIS+ group compared to controls, but this difference is seen in the hippocampal rather than amygdalar component. As only the amygdala is measured this would obviously go unnoticed.

It is indeed generally the case that in studies in which the amygdala and hippocampus are measured separately, the finding of reduced hippocampal volume in schizophrenia does seem more resilient.[41] Indeed, Vita *et al* go so far as to suggest that of the two structures the hippocampus may be affected earlier in the course of the disease. Despite this however, a number of studies do indeed show substantially reduced amygdala volume relatively early in the course of the disease.[34,51] Reconciling these disparate findings will be difficult, but it is likely that this confusion reflects one of the major problems with structural imaging studies focusing on small structures such as the amygdala; namely, as discussed in the introduction, volume losses associated with conditions such as schizophrenia are at the limits of resolution of current imaging techniques. Separation of the amygdala from the hippocampus results in an even smaller structure, compounding the problem, and making identification of volume differences in the study group even harder to detect.

Considering all the data, it seems likely that both the amygdala and hippocampus are reduced in volume in schizophrenia both in learning disabled and non-learning disabled individuals. Whether there are any abnormalities of amygdala volume prior to disease onset is much more difficult to clarify.

5.2.5 At risk have larger amygdalae premorbidly, but this reduces at disease onset.

Related to the above point is the possibility that though amygdala volume is reduced in established cases of schizophrenia, this is not the case before the individual has developed psychosis. In short, amygdala size reduces at the time of illness onset, possibly reflecting a process associated with disease onset that occurs at this point in schizophrenia aetiology. It may even be that prior to the point of volume reduction, an increase in amygdala volume is seen, reflecting increased activity in this structure, or a protective response to prevent decline into psychosis. Indeed, this could potentially fit with psychopathology experienced at this time. Further findings from the EHRS are that anxiety symptoms are a prominent feature in the prodromal period prior to illness development, and that they may diminish with onset of psychosis.[166] Though obviously non-specific, it was identified as one of the earliest features predictive of schizophrenia. Other investigators have commented on anxiety being pathologically absent in the chronic stages of schizophrenia,[161] though it has been argued that experience of such emotions continues, despite expression being markedly reduced.[25] Given the purported involvement of the amygdala in the emotions of fear and anxiety,[4] it is intriguing to speculate that this prodromal anxiety may coincide with an increase in amygdala size prior to size reduction occurring with onset of

psychosis. If this was the case, then an association may be expected between anxiety symptoms and amygdala size. Though there is no evidence of this from the data, it may be of course that the group is too small or heterogeneous to reveal such a trend. Though data is sparse, there are studies that have reported an association between larger amygdala volume and anxiety symptoms.[168]

The idea that volume reductions in the AHC occur at the point of transition into schizophrenia does have some support from imaging studies from the Edinburgh High Risk Study. [56,58] It must be noted however that this study clearly identified reduced volume of the AHC before subjects had developed a formal psychotic illness, rather than structures being larger than controls, as seen in this study. The fact that the amygdala and hippocampus were measured as a single entity and that this may obscure differences of amygdala size in high risk subjects and controls at every time point must of course again be emphasised.

5.2.6 The CBCL selects a group with enlarged amygdalae

As discussed above, at the outset of this study it was expected that amygdala volume would be reduced in the CBCL+/SIS+ group compared to controls. This was not found however, amygdala volume actually being larger in the CBCL+/SIS+ group. Indeed, when raw data for the right amygdala were compared this fell just short of significance, and when this comparison was repeated with covariation for IQ, (along with gender and whole brain volume), it actually reached significance. As discussed in Chapter 4, covariation for IQ is probably not appropriate for this study, but nevertheless these findings are striking and require further discussion.

One possible explanation for this unexpected finding comes from studies investigating amygdala abnormalities in children with behavioural disturbance. Of particular note in relation to this is work of Whittle *et al* investigating brain structural correlates with adolescent affective behaviour.[162] As part of a broader study, they measured amygdala volume in a sample of 137 adolescents without a diagnosis of mental illness or learning disability. The relationship between amygdala volume and measures of various behaviours assessed during parent-child interactions was then ascertained. As predicted by the baseline hypothesis, the duration of aggressive behaviours during a conflict resolution interaction with parents was predicted by the volume of the amygdalae; the association with the left amygdala was significant ($P = <0.05$), while the association with the right amygdala fell just short of significance ($P = 0.057$).

As discussed in the introduction, the study group in this report were selected from a population with special educational needs on the basis of scores on the CBCL and SIS. The CBCL is a tool, generally rated by parents, with which children are rated on the levels of various problem behaviours exhibited. These constitute eight 'constructs' or 'syndromes', two of which are delinquent behaviour and aggressive behaviour. For entry into the study group the cut off chosen was 86, which (unsurprisingly given the nature of the population), is higher than the cut off used for caseness in other studies.[169] Given the nature of many of the problems identified by the CBCL, it would be expected that individuals scoring highly on it would also exhibit longer duration of aggressive behaviour during conflict resolutions. In short, groups identified by score on the CBCL and aggressive behaviour during interactions with parents may share some characteristics. If this was the case, then on the basis of

the work of Whittle *et al* there would be a significant association between score on the CBCL and amygdala volume (being particularly the case for left amygdala).

As was seen in Table 3.8, within the CBCL+/SIS+ group the association that may have been expected between score on CBCL and amygdala volume was not seen. Within the control group however the picture is rather different. After controlling for gender and whole brain volume, a significant positive association is seen between score on CBCL and volume of the left amygdala ($P = 0.049$), with this being even more significant on controlling for gender alone ($P = 0.035$). Additionally, when the two groups (CBCL+/SIS+ and controls) are combined and the analysis repeated for all subjects (again controlling for gender and whole brain volume), a significant association is again seen between score on the CBCL and volume of the left amygdala ($P = 0.035$).

From the discussion above it can be seen that there is an association between score on the CBCL and increased amygdala volume, this being statistically significant on the left side. This is compatible with the data reported by Whittle *et al*,[162] and would provide an explanation as to why, in contrast to what had been expected at the outset of the study, amygdala volume was increased in the CBCL+/SIS+ group compared to controls. What is not seen however is an association between score on the CBCL and amygdala volume *within* the study group. On reflection however, I do not feel that this is particularly surprising. Firstly, it is the case that all subjects within this group are scoring highly on this rating scale, thus reducing the spread of scores and reducing the likelihood of a significant finding. Secondly, as discussed above, this is a very heterogeneous group; there are likely numerous influences on amygdala volume, again making a significant association between score on CBCL and amygdala volume unlikely.

5.2 Part 2 - Relationship between score on PANSS and amygdala volume

A significant association was seen between score on the negative symptom category of PANSS and reduced left amygdala size within the study group. This persisted after adjustment for whole brain volume. One possible explanation for it is that individuals undergoing the process of transition to schizophrenia experience a reduction in amygdala volume, this occurring before they fulfil diagnostic criteria for the illness. This process may be manifest clinically as the development of negative symptoms. Indeed, the concept of the schizophrenic prodrome has long been recognised, encapsulating the period when an individual destined to develop schizophrenia becomes more withdrawn and introverted and loses drive, determination and interest.[170] These are clearly negative-type symptoms.

The idea that the period preceding transition to frank psychosis may be associated with structural brain changes is not new. As discussed in section 1.2.5, Lawrie *et al* observed reductions in temporal lobe volume (statistically significant on the right side) in a longitudinal study comparing those who developed transient psychotic symptoms to those who didn't within a high risk population.[58] The Melbourne group have also reported longitudinal temporal lobe grey matter changes, in the case of their study over the period of transition from prodrome to frank psychosis.[55]

An alternative explanation for the association of greater weight of negative symptoms with smaller amygdala is that the association is static in time, i.e. there is a subgroup within the CBCL+/SIS+ cohort with a longstanding co-occurrence of smaller amygdala and more negative symptoms. This is supported by the absence of a significant association between age and amygdala volume in the study group. Given

that the data are from a single time-point however, it is impossible to be certain if this is a trait or state association. The possibility and implications of this association being a trait or state characteristic will be discussed further below.

Smaller amygdala were associated with greater weight of negative symptoms on both the right and left sides. This association was much more marked on the left however, and was only statistically significant on this side. Considered with the findings from studies of amygdala volume in established schizophrenia this is itself an interesting finding. As noted in section 1.6, in studies in which smaller amygdala are seen only unilaterally, this is almost invariably on the left side. Studies with unilateral findings tend to be first episode studies, which may suggest that left amygdala volume loss occurs earlier in the course of schizophrenia than right. If this was indeed the case, then this would fit with the findings of this study. If the subjects within the study group with a higher weight of negative symptoms were indeed beginning the process of transition to schizophrenia, they would be expected to exhibit the structural correlates of this; they would be expected to have smaller left amygdala.

Findings relevant to the discussion of laterality of amygdala volume loss are also apparent from the studies of Sanderson *et al* and Moorehead *et al* of schizophrenia in learning disability.[104,114] In the VBM analysis of this data, smaller amygdala in comorbids compared to controls was seen only on the left side.[114] It is interesting that once again in this study it is the left side which shows the strongest association between volume reduction and greater weight of negative symptoms. This would be compatible with a model of schizophrenia in which the left amygdala rather than the right shows the most marked volume loss.

5.3 Part 3 - Relationship between age and amygdala volume in the study and control groups.

The relationship between age and amygdala volume was determined in both the study and control groups. In both groups there was a non-significant, negative relationship between increasing age and left amygdala volume. This relationship was slightly stronger in the study group compared to controls, but the difference in the relationship between the two groups still fell far short of significance.

The relationship between amygdala volume and age seen in the control group is compatible with the existing data addressing amygdala volume changes with age during the period of adolescence/early adulthood. This data was discussed in Section 5.1. In contrast, the lack of a significant association between amygdala volume and age within the study group is more surprising. As discussed above, it is being suggested that the association between reduced amygdala volume and greater weight of negative symptoms represents the beginning of a process of transition to schizophrenia; if this is the case however then older subjects within the study group would be expected to have advanced further along this course and thus have smaller amygdalae. A significant negative association between age and amygdala volume would thus be seen; this is not the case. Potential explanations for the absence of this finding are discussed below.

5.4 Part 4 - Reconciling the relationship between score on PANSS and age within the study group and absence of a significant trend towards greater amygdala volume loss with age in this group.

A significant association was seen between score on both the negative and disorganisation symptom categories of PANSS and age within the study group, i.e. the older an individual within this particular high risk group is, the greater the chance they will exhibit disorganisation and negative symptoms. As an isolated finding this would not support a static explanation for the association between greater negative symptoms and smaller amygdala size, but would be consistent with the hypothesis that there is a process occurring within the study group that is resulting in them accruing negative symptoms and losing amygdala volume.

The above finding must be considered together with data comparing the relationship between amygdala volume and age in the study and control groups. As described above, in both groups there was a non-significant, negative relationship between increasing age and left amygdala volume. This relationship was not significantly greater in the study group than the control group. Given the relationship between age and negative symptoms, this result is somewhat surprising. If it is postulated that a process of transition to schizophrenia is occurring in the study group, and this is manifest as a loss of volume and accumulation of negative symptoms, then it would be expected that there would be a more marked association between increasing age and smaller amygdala volume in the high risk group. Clearly, this is not seen.

A number of possible explanations can be proposed to explain the absence of this association. These are outlined below:

5.4.1 Anomalous nature of control data

This explanation is unlikely. As discussed above the association between amygdala volume and age within the control group is compatible with existing data.[73]

5.4.2 *Lack of statistical power*

A definite trend was seen towards increasing age being associated with reduced volume of the left amygdala, the *P* value being 0.106. It is conceivable that had the study group been larger, this would reach significance.

5.4.*3 Heterogeneity within study group*

An alternative explanation could stem from the heterogeneous nature of the CBCL+/SIS+ group. It could be argued that though an association between increasing age and smaller amygdala volume does exist within a subset of the group, it is being masked by another subset in which there is a different relationship between amygdala volume and age.

5.4.4 Dynamic processes occurring in amygdalae of study group

If the period preceding development of schizophrenia was characterised first by enlargement of the amygdala and then volume loss, then it would be unlikely that overall a relationship between age and loss of volume would be seen. If a period of anxiety associated with increased amygdala volume was followed by volume loss associated with negative symptoms, then it can be seen how this lack of an association between smaller volume and age could co-exist with an association between greater weight of negative symptoms and increasing age. There is indeed some data supporting an association between increased amygdala volume and anxiety symptoms.[168]

5.4.5 The association between smaller left amygdala and greater weight of negative symptoms is a trait characteristic

Despite the discussion above, it must also be acknowledged that absence of a significant difference in the rate of volume loss with age in the study group compared to controls may best fit with a static explanation for the association between more negative-type symptoms and smaller amygdala. This would suggest that within a group of learning disabled subjects shown by other measures to be at high risk for schizophrenia there is a subgroup who display both relatively smaller amygdalae and a greater weight of negative symptoms, this being a trait association. Given that the basic premise for this study was that there may be a subgroup of learning disabled individuals whose cognitive impairment is due to a schizophrenic illness yet to

become manifest as psychotic symptoms, this is an intriguing possibility. It may be that within this group those individuals with relatively smaller amygdalae and more negative symptoms are at even greater risk of developing schizophrenia than the group as a whole. As and if illness develops it would be expected that there would be further volume reduction at that time point, associated with worsening of negative symptomatology and emergence of frank psychotic symptoms. Clearly follow up will be required to determine if these characteristics truly do identify an ultra-high risk group. If this is the case however, then it may be that these are further characteristics which can be used to refine models designed to predict the risk of schizophrenia developing in a learning disabled individual.

5.4.6 Increase in weight of negative symptoms with age is due to a process in which the amygdala is not of primary importance.

It is clear that within the study group there is a strong association between smaller left amygdala volume and greater weight of negative symptoms. It is also clear that the strength of this association increases after covariation for age. Thus, it is not simply that age is a confounding factor. It is also the case however that given that amygdala volume does not seem to decline significantly with age in the study group, loss of amygdala volume in a pre-schizophrenic population is unlikely a continuous, progressive process. Thus, while a baseline increase in negative symptoms may well be related to amygdala volume loss, as time progresses changes in other parts of the brain may be more important in worsening of this sympomatology.

On considering the above, it can be seen that there is commonality with the two-hit hypothesis of schizophrenia. One interpretation of this model, as proposed by Karachi, is that temporal lobe changes may underlie a vulnerability to schizophrenia, with latent dysfunction in these regions becoming clinically apparent as positive symptoms due to additional frontal lobe changes.[171] The inter-relationship of these two brain regions and its potential importance in schizophrenia is also discussed. Of particular note they cite data providing evidence of a significant relationship between the volume of the prefrontal lobe and the volume of temporal lobe structures in patients with schizophrenia, this not being present in healthy controls.[171] This would support the hypothesis of a dynamic interaction between these two brain regions being important in the aetiology of schizophrenia.

Interpreting this model in light of my data, it is conceivable that relatively smaller amygdalae in individuals at high risk for schizophrenia reflect structural abnormalities of the region. Within a pre-illness group such as this, these abnormalities may give rise to a degree of negative symptomatology. As further changes occur however, possibly as a consequence of additional frontal lobe changes, frank psychotic symptoms will emerge, negative symptomatology worsen and a schizophrenic illness diagnosed.

5.5 Part 5 - Relationship between amygdala volume and score on positive symptom subset of PANSS

No association was seen between size of either left or right amygdala and the positive symptom subset of PANSS. A number of explanations can be proposed for this finding. One possibility, as alluded to above, is that while negative symptoms arise from amygdala abnormalities, other structures are more important in positive symptoms. Alternatively, it may be that early and possibly longstanding changes in amygdala structure are important in negative symptoms, while later changes, reflected in further volume loss, are more important in positive symptoms. This does not deny the importance of the amygdala in these later symptoms; it simply means that as individuals in this population who are destined to develop schizophrenia are early in this process, the changes associated with positive symptoms would not be expected yet. Alternatively, it may be that amygdala changes are indeed important in the manifestation of schizophrenia, and this process has occurred, but another part of the brain is compensating for abnormalities of amygdala function at this timepoint. As the compensating structures become less able to perform this function, positive symptoms of schizophrenia may develop. This would fit with data from the Pittsburgh high risk study which was discussed earlier; though AHC volume reductions were seen in this population, reductions of the dorso-lateral prefrontal cortex were not.[61]

5.6 Part 6 - Relevance of autism data to the relationship between amygdala size and score on the negative symptom subset of PANSS

There is a further body of research relevant to discussion of the relationship between amygdala volume and score on the negative symptom subset of PANSS. This is research investigating amygdala volume in autism.

It is well recognised that there are similarities between the autistic spectrum disorders and schizophrenia, and that this is particularly the case for the negative symptom subset of the latter condition. Indeed, the extent to which these clinical presentations may overlap has been formally researched. In a study of 21 individuals with chronic, treatment resistant schizophrenia Sheitman *et al* found that autistic features as measured by the Autism Behaviour Checklist correlated with the degree of negative but not positive symptoms.[172] Examining the issue from the opposite direction Konstantareas et al found that 50% of autistic individuals met diagnostic criteria for schizophrenia, disorganised type with negative symptoms.[173] In a direct comparison of autism and schizophrenia the autistic subjects were found to have significantly less positive thought disorder than schizophrenic subjects but with no difference in measures of affective flattening.[174] These findings indicate that negative symptoms are shared between the disorders but that autistic individuals do not show the same degree of positive symptomatology as is found in schizophrenia. There are two possibilities which could explain this commonality; either negative symptoms of schizophrenia and autistic features are different phenomena which are simply difficult to distinguish clinically, or they represent the same underlying pathophysiogical process. If the first possibility is true, then it is indeed possible that some subjects on the autistic spectrum were included within the CBCL+/SIS+ group.

It has been reported that, compared to controls, the amygdala is enlarged in children but not adolescents with autism.[128] Indeed, in autistic adults it may even be smaller than that of control subjects.[175,176] It thus seems likely that though in younger children the amygdala is larger in autistic than normally developing subjects, in older children there is little difference in size. Thus, given that the CBCL+/SIS+ cohort is an adolescent group, if there were autistic individuals in it, these individuals would tend to have similar size amygdalae to control subjects. The very youngest may be expected to have marginally larger amygdalae, and the oldest marginally smaller. In the group as a whole however, it would be expected that there would be no significant difference between amygdala volumes. This is of course what is seen. As autistic symptoms would not be expected to increase with age, the association between increasing age and greater weight of negative symptoms would not be expected if these symptoms were indeed due to autism. This argues against autistic traits accounting for elevated score on the PANSS.

5.7 Part 7 - Integration of findings into broader knowledge base of aetiology of schizophrenia

There are two core findings from this study. The first is that, contrary to what may have been expected, when compared to controls there was no evidence for reduced amygdala volume in a learning disabled population with no history of psychosis, but at high risk for developing schizophrenia. Indeed, though it fell just short of statistical significant, mean amygdala volume was actually larger than in the control population. The second is that within the population at high risk for

schizophrenia, greater weight of negative symptoms was associated with smaller amygdala volume; this association was statistically significant. The question of whether this is a trait characteristic or represents the beginning of a process of transition to schizophrenia cannot be adequately answered on the basis of this cross-sectional study. If it is the case that further study of this group reveals further loss of amygdala volume and this correlates with a further increase in weight of negative symptoms then it would of course favour the latter. Regardless of what happens in the future however, it is the case that this association exists at present. Given this it is important to appreciate how these two findings integrate with other published findings from the comorbidity study, the broader knowledge base for the aetiology of schizophrenia and what is known about the role of the amygdala in mental and behavioural disorders in general.

5.7.1 Integration of current findings with previous publications from this study group

The principle publications of relevance to this report that have arisen from the comorbidity study are those of Spencer *et al*. Spencer's 2007 paper discussed grey matter correlates of early psychotic symptoms in adolescents at elevated risk of psychosis.[177] It is important to underline that the group under investigation in this study was selected on the basis of receiving additional learning support; they had not been selected for enhanced vulnerability to schizophrenia on the basis of the SIS and CBCL. Thus though they did have the three fold elevation of risk conferred by being learning disabled, they would be expected to be at significantly less mean risk than the population in this study. Findings included a significant negative correlation

between anxiety and grey matter density of the left hippocampus. No significant associations between specific symptoms and amygdalar density are reported.

5.7.2 Integration of current findings with the broader knowledge base for aetiology of schizophrenia

It is clear that cognitive dysfunction is a cardinal feature of schizophrenia, and the condition is widely regarded as a neurocognitive disorder. As discussed in the introduction however, there has also been substantial interest in the importance of emotional brain systems in the disorder, which may transpire to be as important. As the amygdala is viewed to be central to emotional experience, learning and memory,[25,178] it is thus unsurprising that significant attention has focussed on this structure. It is important that an attempt is made to integrate the findings discussed above into a model addressing the neurobiology of emotional processing in schizophrenia.

In their review, Alemann and Kahn attempt to explain the emotional abnormalities characteristic of schizophrenia by proposing a model in which (1) a dopamine imbalance underlies the increased emotional experience associated with psychosis, whereas (2) structural volume reductions of the amygdala and reduced connectivity with the prefrontal cortex underlie the emotion perception deficit and the reduction in emotional expressive behaviour.[25] Elevated levels of dopamine would be important in the former, whereas structural lesions of the amygdala would give rise to the latter. They stress that the focus on a lesion in the amygdala can explain the

'emotion paradox' of schizophrenia, whereby deficient recognition of emotion expressions and social perception coexists with largely intact emotional experience.

When discussing neural mechanisms underlying the negative symptoms of schizophrenia, Alemann and Kahn refer to the model proposed by Grossberg.[179] The essence of this model is that emotional centres of the brain, in particular the amygdala, interact with sensory and prefrontal cortices to generate affective states and elicit motivated behaviours. If emotional centres become depressed, feedback loops are disturbed and negative symptoms emerge. Thus, a primary lesion in the amygdala can have widespread effects. Specifically, it is suggested that one possible cause of decreased prefrontal activity in schizophrenia may be a reduction in incentive motivating signals from depressed amygdala circuits that project to the prefrontal cortex.

Though Grossberg's model can go some way to explain the occurrence of negative symptoms in schizophrenia, it does not account for positive symptoms; these are, of course, generally the most striking manifestations of the illness. A model which can potentially account for the genesis of these symptoms, the process of 'aberrant assignment of salience' to otherwise insignificant stimuli, was discussed in Section 1.1.[25,180] It is important to recap and expand on this model at this point.

Kapur's model was built on theories of the role of mesolimbic dopamine in the healthy brain.[181] In this context it is argued that mesolimbic dopamine acts to provide significance or salience, transforming an affectively neutral stimulus into an attractive or aversive one.[182] Thus, mesolimbic dopamine activity may determine whether an intrusion into awareness from either external perceptual or internal mental sources receives a positive or negative 'hedonic vector' and thus 'grabs the attention' of the individual. If psychosis were associated with increased, stimulus-independent, release

of dopamine, salience would be granted to what would otherwise be relatively innocuous events or stimuli.[181] This process has been succinctly described as dopamine providing 'the wind to the psychotic fire'.[183]

Such a theory of 'aberrant assignment of salience' integrates well with a model of emotional hyperarousal advanced by Grace. In this model the amygdala is assumed to play an important role in a circuit including prefrontal pathways to the nucleus accumbens.[25] The nucleus accumbens is a structure considered central to motivated behaviour, drive and salience[184] and is widely regarded to be an important site for antipsychotic drug action.[25] Within this model, according to Grace, the prefrontal cortex provides multiple motor plans by which it drives goal-directed behaviour.[185] The most effective plan is then selected within the nucleus accumbens via the facilitatory effects of hippocampal and amygdalar infuences. In normal health interplay between the hippocampus and the amygdala helps to maintain an individual in emotional balance. The hippocampus, with input from the prefrontal cortex, sets environmental stimuli in the context of the current situation or past experience of the stimulus, and only response patterns that are appropriate to a given context are allowed to impact on mesolimbic dopamine. In the case of a high affect value stimulus (e.g. implying threat to the organism), the amygdala can over-ride the hippocampal influence to direct behaviour in order to immediately and effectively deal with the challenge. In schizophrenia, according to the model, due to an imbalance in dopamine systems, the amygdala becomes hyperactive and not only fails to facilitate prefrontal cortical throughput, but actually competes with it for driving nucleus accumbens activity. The system thus responds inappropriately to otherwise insignificant stimuli (which acquire salience), leading ultimately to paranoia and psychosis.[25,181]

In the models outlined above, it is being suggested that abnormalities of amygdala function can account for both the positive and negative symptoms of schizophrenia. In the case of positive symptoms this involves overactivity, while negative symptoms are due to underactivity. Such a model may superficially seem to lack coherence, but conceptualisation of the amygdala as a heterogeneous, multifunctional structure can reconcile this apparent contradiction. This issue is further addressed in the review of Aleman *et al*.

Central to the hypothesis that amygdalar abnormalities can account for both constellations of symptoms is evidence concerning the function of two particular regions of the amygdala. These two regions are the basolateral nucleus (BLA) and central nucleus (CeA). The former is believed to play a role in forming associations between sensor stimuli and biologically significant events that have emotional and motivational valence.[184] Output from this region flows to motor circuits of the striatum and cortex (allowing active responding to emotionally arousing stimuli), but also to the CeA. The CeA activates brain stem areas involved in controlling specific involuntary components of emotional reaction, e.g. autonomic and endocrine responses.[25] Building on this model, it is proposed that in schizophrenia there may be a lesion to the BLA, which would cause deficiencies in processing emotional stimuli and emotional learning; the existence of such deficits were discussed in Section 1.1. Additionally this deficit, likely combined with reduced prefrontal connectivity, would also lead to a reduction in active emotional responses, manifesting as negative-type symptoms such as emotional flattening and withdrawal. It is also proposed that such a lesion could have consequences for the input to the CeA. Reduced prefrontal control of the BLA may lead to aberrant activity of the CeA, autonomic arousal and subjective anxiety.[25]

The above model also dovetails neatly with the hypothesis of aberrant assignment of salience being central to the genesis of positive symptoms. It has been suggested by Philips *et al* that the CeA and BLA exert different influences on the mesocorticolimbic dopamine system.[184] Within this model, the CeA maintains control of tonic activity of ventral tegmental dopamine neurones serving the nucleus accumbens and prefrontal cortex. If inhibitory control of the CeA is reduced, then this would result in increased tonic dopamine levels within this system. This would then result in the assignment of emotional salience to otherwise insignificant stimuli.[25]

On considering the models outlined above, the relevance of findings from this study are potentially significant. From the above it would be expected that if an individual experienced a reduction in incentive motivating signals to the prefrontal cortex, these would be clinically manifest as negative type symptoms. As discussed in section 5.2, negative-type symptoms can precede diagnosis of schizophrenia by a substantial period of time, and could be present in the absence of frank psychosis. Thus, in a population at elevated risk of schizophrenia but not yet psychotic, a greater weight of negative symptoms could reflect greater impairment of incentive motivating symptoms from the amygdala. As described by Aleman, this impairment of amygdala function would be expected to have been initiated by a lesion to the structure, particularly the BLA.[25] Given this, it seems logical to expect that the greater the extent of the lesion to the amygdala, the greater the impairment of this function, and thus the greater the weight of negative symptoms. Ideally volume changes in particular regions of the amygdala would be evaluated separately, but unfortunately this is beyond the resolution of current imaging techniques. Thus, only gross amygdala volume can be determined, but from the above this would be expected to

have an inverse relationship with the weight of negative symptoms experienced. In terms of the left amygdala, this is indeed what is seen in this study.

Appendix

Protocol for amygdala measurement

Protocol was based on that of Schumann *et al*.[128] However given that the neuroanatomy of many of the special needs group differed markedly from that of the 'typical' brain, this was modified slightly to further minimise reliance on landmarks external to the amygdale. This is necessary to minimise the possibility of systematic error being introduced. It was clear from a pilot study employing a provisional protocol that if fixed landmarks were used, even if they surrounded the amygdala, then there would be times when one would be including what was clearly empty space in the delineation of the amygdale. For this reason a rule of being generally conservative, and only tracing what was clearly present was employed, rather than extending the amygdala tracing to where it would generally be bounded by a landmark. This principle of tracing conservatively was also employed in other circumstances, such as when boundaries with white matter were indistinct.

<u>General tracing considerations</u>

Tracing is carried out initially in the coronal view. However some borders are much more easily seen in the sagittal view, and sometimes the axial view. The sagittal view is particularly useful in the establishment of the posterior and anterior borders. Superior and inferior should be examined on coronal and sagittal.
All tracing was undertaken with frequent reference to an atlas of neuroanatomy to further ensure accuracy of anatomical delineation.[186]

General points about tracing protocol

As mentioned above, tracing begins in coronal view, progressing through slices caudally to rostrally. The amygdala emerges superolaterally to hippocampus. It appears on the same slice as the hippocampus for approximately 3-5 slices, moving inferomedially to encompass the hippocampal head on three sides- medial, lateral and superior. It becomes indistinct in anterior slices; this means that the anterior border requires to be established almost entirely in the saggital view.

To further increase consistency between scans the contrast setting of 20/130 was used for all tracing. This setting could be adjusted to aid viewing of structures, but was fixed for time of tracing

First slice

Appears superolateral to the hippocampus. Begin tracing here, though it will be edited in sagittal view. The borders are:

Superior: putamen and optic tract

Inferior: temporal horn of lateral ventrical, or hippocampus or alveus

Lateral: putamen (usually border follows white matter or may need to be extrapolated)

Moving rostrally

Amygdala moves to encompass and then replace the hippocampus. The borders are:

Superior: laterally the putamen, medially the medial surface of brain

Medial: medial surface of brain

Inferior: temporal horn of lateral ventricle, or hippocampus or alveus

Lateral: white matter of temporal stem.

In these slices the principle of conservative tracing is particulary crucial. The lateral and medio-lateral borders are particularly indistinct, as the point where white matter begins is frequently not clear. To avoid inclusion of the ventral claustrum in particular, caution was paid not to extend the outline of the amygdala into the temporal stem.

Then, on reaching the more rostral portion of the amygdala the borders are:

Superior: medial surface of brain

Medial: medial surface of brain

Infeior and inferomedial: white matter of entorhinal cortex. In practice, this white matter does not extend all the way to the medial surface however, and needs to be extrapolated. If the semiannular sulcus is visible on the medial surface, then this line is drawn from the most medial point of the enterorhinal white matter to it. If the semiannular sulcus is not seen, then a line of best fit is drawn. Interpolation between slices may assist with this. This is an area which can be considerably revised in the sagittal view, as the white matter white matter of the entorhinal cortex is frequently more visible in it.

Lateral: white matter of temporal stem; again this can be indistinct, and a general rule of conservative tracing is followed.

Most rostral portion of the amygdala

At this point the boundaries between grey and white matter become even more difficult to delineate. At the most rostral point of the amygdala this becomes impossible, necessitating confirmation of the rostral boundary of the amygdala by review of sagittal images.

Revision in sagittal view

Scroll from lateral to medial. Particular areas are consistently likely to need revision.

1) Posterior border with the hippocampus.

This is generally easily seen in the sagittal view. There is a thin strip of grey matter which protrudes posteriorly from the superior edge of the amygdala; this is the hippocampal-amygdala transitional are (HATA), defined by Convit *et al*.[153] It is edited out.

2) Anterior border

Difficult to see in any view. The white matter separating amygdala from entorhinal cortex is frequently more visible in the sagittal view.

3) Superior border

White matter and optic tract act as borders. This can assist with separation from the putamen. Again the principle of conservative tracing was followed

References

1. Suslow, T., et al. The experience of basic emotions in schizophrenia with and without affective negative symptoms. *Comprehensive psychiatry* 44.4 (2003): 303-310
2. Kluver, H. and P.C. Bucy. 'Psychic blindness' and other symptoms following bilateral temporal lobectomy in rhesus monkeys. *American Journal of Physiology* 119 (1937): 352-53.
3. Weiskrantz, L. Behavioral changes associated with ablation of the amygdaloid complex in monkeys. *J.Comp Physiol Psychol.* 49.4 (1956): 381-91.
4. Aggleton, JP. The Amygdala, Second Edition, A Functional Analysis. Oxford: Oxford University Press, 2000. 213-87.
5. Applegate, C.D., et al. Autonomic and somatomotor effects of amygdala central N. stimulation in awake rabbits. *Physiol Behav.* 31.3 (1983): 353-60.
6. Bechara, A., et al. Double dissociation of conditioning and declarative knowledge relative to the amygdala and hippocampus in humans. *Science* 269.5227 (1995): 1115-18.
7. Powell, D.A., et al. Amygdala-prefrontal interactions and conditioned bradycardia in the rabbit. *Behav.Neurosci.* 111.5 (1997): 1056-74.
8. McCabe, P.M., et al. Ibotenic acid lesions in the amygdaloid central nucleus but not in the lateral subthalamic area prevent the acquisition of differential Pavlovian conditioning of bradycardia in rabbits. *Brain Res.* 580.1-2 (1992): 155-63.
9. Miserendino, M.J., et al. Blocking of acquisition but not expression of conditioned fear-potentiated startle by NMDA antagonists in the amygdala. *Nature* 345.6277 (1990): 716-18.
10. Quirk, G.J. and Gehlert, D.R. Inhibition of the amygdala: key to pathological states? *Ann.N.Y.Acad.Sci.* 985 (2003): 263-72.
11. Gabriel, M., Burhans, L., and Kashef, A. Consideration of a unified model of amygdalar associative functions. *Ann.N.Y.Acad.Sci.* 985 (2003): 206-17.
12. Adolphs, R. Is the human amygdala specialized for processing social information? *Ann.N.Y.Acad.Sci.* 985 (2003): 326-40.
13. Whalen, P.J., et al. Masked presentations of emotional facial expressions modulate amygdala activity without explicit knowledge. *J.Neurosci.* 18.1 (1998): 411-18.
14. Phillips, M.L., et al. A specific neural substrate for perceiving facial expressions of disgust. *Nature* 389.6650 (1997): 495-98.
15. Rosvold, H.E., Mirsky, A.F. and Pribram, K.H. Influence of amygdalectomy on social behavior in monkeys. *J.Comp Physiol Psychol.* 47.3 (1954): 173-78.
16. Adolphs, R., Tranel, D. and Damasio, A.R. The human amygdala in social judgment. *Nature* 393.6684 (1998): 470-74.
17. Fine, C., Lumsden, J. and Blair, R.J. Dissociation between 'theory of mind' and executive functions in a patient with early left amygdala damage. *Brain* 124.Pt 2 (2001): 287-98.
18. Barton, R.A. and Aggleton, J.P. Primate Evolution and the Amygdala. In: The Amygdala, Second Edition, A Functional Analysis. (ed. J.P. Aggleton) 2000. pp. 479-508. Oxford University Press: Oxford.
19. Adolphs, R., Baron-Cohen, S. and Tranel, D. Impaired recognition of social emotions following amygdala damage. *J.Cogn Neurosci.* 14.8 (2002): 1264-74.

20. Bell, M., Bryson, G. and Lysaker P. Positive and negative affect recognition in schizophrenia: a comparison with substance abuse and normal control subjects. *Psychiatry Res.* 73.1-2 (1997): 73-82.
21. Bellack, A.S., Blanchard, J.J and Mueser K.T. Cue availability and affect perception in schizophrenia. *Schizophr.Bull.* 22.3 (1996): 535-44.
22. Shayegan, D.K. and Stahl, S.M. Emotion processing, the amygdala, and outcome in schizophrenia. *Prog.Neuropsychopharmacol.Biol.Psychiatry* 29.5 (2005): 840-45.
23. Hall, J., et al. Social cognition and face processing in schizophrenia. *Br.J.Psychiatry* 185 (2004): 169-70.
24. Pinkham, A.E., et al. Implications for the neural basis of social cognition for the study of schizophrenia. *Am.J.Psychiatry* 160.5 (2003): 815-24.
25. Aleman, A. and Kahn, R.S. Strange feelings: do amygdala abnormalities dysregulate the emotional brain in schizophrenia? *Prog.Neurobiol.* 77.5 (2005): 283-98.
26. Taylor, S.F., et al. A functional anatomic study of emotion in schizophrenia. *Schizophr.Res.* 58.2-3 (2002): 159-72.
27. Potkin, S.G., et al. A PET study of the pathophysiology of negative symptoms in schizophrenia. Positron emission tomography. *Am.J.Psychiatry* 159.2 (2002): 227-37.
28. Brothers, L. The social brain: a project for integrating primate behaviour and neurophysiology in a new domain. *Concepts Neurosci* 1, 27-51. 1990.
29. Johnstone, E.C., et al. Cerebral ventricular size and cognitive impairment in chronic schizophrenia. *Lancet* 2.7992 (1976): 924-26.
30. Harrison, P.J. The neuropathology of schizophrenia. A critical review of the data and their interpretation. *Brain* 122 (Pt 4) (1999): 593-624.
31. Job, D.E., et al. Structural gray matter differences between first-episode schizophrenics and normal controls using voxel-based morphometry. Neuroimage. 17.2 (2002): 880-89.
32. Shenton, M.E., et al. A review of MRI findings in schizophrenia. *Schizophr.Res.* 49.1-2 (2001): 1-52.
33. Lawrie, S.M. and Abukmeil, S.S. Brain abnormality in schizophrenia. A systematic and quantitative review of volumetric magnetic resonance imaging studies. *Br.J.Psychiatry* 172 (1998): 110-20.
34. Wright, I.C., et al. Meta-analysis of regional brain volumes in schizophrenia. *Am.J.Psychiatry* 157.1 (2000): 16-25.
35. Lawrie, S.M., et al. Magnetic resonance imaging of brain in people at high risk of developing schizophrenia. *Lancet* 353.9146 (1999): 30-33.
36. Velakoulis, D., et al. Hippocampal volume in first-episode psychoses and chronic schizophrenia: a high-resolution magnetic resonance imaging study. *Arch.Gen.Psychiatry* 56.2 (1999): 133-41.
37. Warwick, M.M., et al. Volumetric magnetic resonance imaging study of the brain in subjects with sex chromosome aneuploidies. *J.Neurol.Neurosurg.Psychiatry* 66.5 (1999): 628-32.
38. Shenton, M.E., et al. A review of MRI findings in schizophrenia. *Schizophr.Res.* 49.1-2 (2001): 1-52.
39. Gur, R.E., et al. Temporolimbic volume reductions in schizophrenia. *Arch.Gen.Psychiatry* 57.8 (2000): 769-75.
40. Suzuki, M., et al. Differential contributions of prefrontal and temporolimbic pathology to mechanisms of psychosis. *Brain* 128.Pt 9 (2005): 2109-22.

41. Vita, A., et al. Brain morphology in first-episode schizophrenia: a meta-analysis of quantitative magnetic resonance imaging studies. *Schizophr.Res.* 82.1 (2006): 75-88.
42. Steen, R.G., et al. Brain volume in first-episode schizophrenia: systematic review and meta-analysis of magnetic resonance imaging studies. *Br.J.Psychiatry* 188 (2006): 510-18.
43. Joyal, C.C., et al. The amygdala and schizophrenia: a volumetric magnetic resonance imaging study in first-episode, neuroleptic-naive patients. *Biol.Psychiatry* 54.11 (2003): 1302-04.
44. Honea, R., et al. Regional deficits in brain volume in schizophrenia: a meta-analysis of voxel-based morphometry studies. *Am.J.Psychiatry* 162.12 (2005): 2233-45.
45. Hulshoff Pol, H.E., et al. Focal gray matter density changes in schizophrenia. *Arch.Gen.Psychiatry* 58.12 (2001): 1118-25.
46. Thompson, P.N. et al. Automated Analysis of Structural MRI Data. Schizophrenia: From neuroimaging to neuroscience. Ed. S Lawrie, EC Johnstone, and D Weinberger. Oxford: Oxford University Press, 2004. 119-65.
47. DeLisi, L.E. and Hoff, A.L. Failure to find progressive temporal lobe volume decreases 10 years subsequent to a first episode of schizophrenia. *Psychiatry Res.* 138.3 (2005): 265-68.
48. Pantelis, C., et al. Structural brain imaging evidence for multiple pathological processes at different stages of brain development in schizophrenia. *Schizophr.Bull.* 31.3 (2005): 672-96.
49. Gur, R.E., et al. A follow-up magnetic resonance imaging study of schizophrenia. Relationship of neuroanatomical changes to clinical and neurobehavioral measures. *Arch.Gen.Psychiatry* 55.2 (1998): 145-52.
50. Kasai, K., et al. Progressive decrease of left Heschl gyrus and planum temporale gray matter volume in first-episode schizophrenia: a longitudinal magnetic resonance imaging study. *Arch.Gen.Psychiatry* 60.8 (2003): 766- 75.
51. James, A.C., et al. Evidence for non-progressive changes in adolescent-onset schizophrenia: follow-up magnetic resonance imaging study. *Br.J.Psychiatry* 180 (2002): 339-44.
52. Whitworth, A.B., et al. Longitudinal volumetric MRI study in first- and multiple-episode male schizophrenia patients. *Psychiatry Res.* 140.3 (2005): 225-37.
53. Velakoulis, D., et al. Hippocampal and amygdala volumes according to psychosis stage and diagnosis: a magnetic resonance imaging study of chronic schizophrenia, first-episode psychosis, and ultra-high-risk individuals. *Arch.Gen.Psychiatry* 63.2 (2006): 139-49.
54. Altshuler, L.L., et al. Amygdala enlargement in bipolar disorder and hippocampal reduction in schizophrenia: an MRI study demonstrating neuroanatomic specificity. *Arch.Gen.Psychiatry* 55.7 (1998): 663-64.
55. Pantelis, C., et al. Neuroanatomical abnormalities before and after onset of psychosis: a cross-sectional and longitudinal MRI comparison. *Lancet* 361.9354 (2003): 281-88.
56. Lawrie, S.M., et al. Structural and functional abnormalities of the amygdala in schizophrenia. *Ann.N.Y.Acad.Sci.* 985 (2003): 445-60.
57. Job, D.E., et al. Voxel-based morphometry of grey matter densities in subjects at high risk of schizophrenia. *Schizophr.Res.* 64.1 (2003): 1-13.

58. Lawrie, S.M., et al. Temporal lobe volume changes in people at high risk of schizophrenia with psychotic symptoms. *Br.J.Psychiatry* 181 (2002): 138-43.
59. Lawrie, S.M., et al. Structural and functional abnormalities of the amygdala in schizophrenia. *Ann.N.Y.Acad.Sci.* 985 (2003): 445-60.
60. Job, D.E., et al. Grey matter changes over time in high risk subjects developing schizophrenia. *Neuroimage.* 25.4 (2005): 1023-30.
61. Keshavan, M.S., et al. Decreased left amygdala and hippocampal volumes in young offspring at risk for schizophrenia. *Schizophr.Res.* 58.2-3 (2002): 173-83.
62. Schneider, F., et al. Differential amygdala activation in schizophrenia during sadness. *Schizophr.Res.* 34.3 (1998): 133-42.
63. Phillips, M.L., et al. A differential neural response to threatening and non-threatening negative facial expressions in paranoid and non-paranoid schizophrenics. *Psychiatry Res.* 92.1 (1999): 11-31.
64. Fahim, C., et al. Brain activity during emotionally negative pictures in schizophrenia with and without flat affect: an fMRI study. *Psychiatry Res.* 140.1 (2005): 1-15.
65. Laurens, K.R., et al. Attention orienting dysfunction during salient novel stimulus processing in schizophrenia. *Schizophr.Res.* 75.2-3 (2005): 159-71.
66. Das, P., et al. Functional disconnections in the direct and indirect amygdala pathways for fear processing in schizophrenia. *Schizophr.Res.* 90.1-3 (2007): 284-94.
67. Holt, D.J., et al. Increased medial temporal lobe activation during the passive viewing of emotional and neutral facial expressions in schizophrenia. *Schizophr.Res.* 82.2-3 (2006): 153-62.
68. Sanjuan, J., et al. Emotional words induce enhanced brain activity in schizophrenic patients with auditory hallucinations. *Psychiatry Res.* 154.1 (2007): 21-29.
69. Brunet-Gouet, E. and Decety, J. Social brain dysfunctions in schizophrenia: a review of neuroimaging studies. *Psychiatry Res.* 148.2-3 (2006): 75-92.
70. Levitt, J.G., et al. Medial temporal lobe in childhood-onset schizophrenia. *Psychiatry Res.* 108.1 (2001): 17-27.
71. Jacobsen, L.K., et al. Temporal lobe morphology in childhood-onset schizophrenia. *Am.J.Psychiatry* 153.3 (1996): 355-61.
72. Jacobsen, L.K., et al. Progressive reduction of temporal lobe structures in childhood-onset schizophrenia. *Am.J.Psychiatry* 155.5 (1998): 678-85.
73. Giedd, J.N., et al. Childhood-onset schizophrenia: progressive brain changes during adolescence. *Biol.Psychiatry* 46.7 (1999): 892-98.
74. Johnstone, E.C. and Owens, D.G. Early Studies of Brain Anatomy in Schizophrenia. Schizophrenia: From neuroimaging to neuroscience. Ed. S Lawrie, E. C. Johnstone, and Weinberger D. Oxford: Oxford University Press, 2004. 1-19.
75. Brown, R., et al. Postmortem evidence of structural brain changes in schizophrenia. Differences in brain weight, temporal horn area, and parahippocampal gyrus compared with affective disorder. *Arch.Gen.Psychiatry* 43.1 (1986): 36-42.
76. Bogerts, B., Meertz, E. and Schonfeldt-Bausch, R. Basal ganglia and limbic system pathology in schizophrenia. A morphometric study of brain volume and shrinkage. *Arch.Gen.Psychiatry* 42.8 (1985): 784-91.

77. Pakkenberg, B. Pronounced reduction of total neuron number in mediodorsal thalamic nucleus and nucleus accumbens in schizophrenics. *Arch.Gen.Psychiatry* 47.11 (1990): 1023-28.
78. Heckers, S., et al. Limbic structures and lateral ventricle in schizophrenia. A quantitative postmortem study. *Arch.Gen.Psychiatry* 47.11 (1990): 1016-22.
79. Chance, S.A., Esiri, M.M. and Crow, T.J. Amygdala volume in schizophrenia: post-mortem study and review of magnetic resonance imaging findings. *Br.J.Psychiatry* 180 (2002): 331-38.
80. Cannon, M. and Jones, P. Schizophrenia. *J.Neurol.Neurosurg.Psychiatry* 60.6 (1996): 604-13.
81. Baare, W.F., et al. Quantitative genetic modeling of variation in human brain morphology. *Cereb.Cortex* 11.9 (2001): 816-24.
82. McIntosh, A and Lawrie, S. Structural Magnetic Resonance Imaging. Schizophrenia: From neuroimaging to neuroscience. Ed. S Lawrie, EC Johnstone, and D Weinberger. Oxford: Oxford University Press, 2004. 21-57.
83. Baare, W.F., et al. Volumes of brain structures in twins discordant for schizophrenia. *Arch.Gen.Psychiatry* 58.1 (2001): 33-40.
84. van Haren, N.E., et al. A controlled study of brain structure in monozygotic twins concordant and discordant for schizophrenia. *Biol.Psychiatry* 56.6 (2004): 454-61.
85. van Erp, T.G., et al. Hippocampal volumes in schizophrenic twins. *Arch.Gen.Psychiatry* 61.4 (2004): 346-53.
86. Staal, W.G., et al. Structural brain abnormalities in patients with schizophrenia and their healthy siblings. *Am.J.Psychiatry* 157.3 (2000): 416-21.
87. Keshavan, M.S., et al. Magnetic resonance imaging and spectroscopy in offspring at risk for schizophrenia: preliminary studies. *Prog.Neuropsychopharmacol.Biol.Psychiatry* 21.8 (1997): 1285-95.
88. Seidman, L.J., et al. Thalamic and amygdala-hippocampal volume reductions in first-degree relatives of patients with schizophrenia: an MRI-based morphometric analysis. *Biol.Psychiatry* 46.7 (1999): 941-54.
89. Schreiber, H., et al. Brain morphology in adolescents at genetic risk for schizophrenia assessed by qualitative and quantitative magnetic resonance imaging. *Schizophr.Res.* 40.1 (1999): 81-84.
90. O'Driscoll, G.A., et al. Amygdala-hippocampal volume and verbal memory in first-degree relatives of schizophrenic patients. *Psychiatry Res.* 107.2 (2001): 75-85.
91. Steel, R.M., et al. Structural MRI of the brain in presumed carriers of genes for schizophrenia, their affected and unaffected siblings. *J.Neurol.Neurosurg.Psychiatry* 72.4 (2002): 455-58.
92. Siever, L J. and Davis, K.L. The pathophysiology of schizophrenia disorders: perspectives from the spectrum. *Am.J.Psychiatry* 161.3 (2004): 398-413.
93. Dickey, C.C., et al. Schizotypal personality disorder and MRI abnormalities of temporal lobe gray matter. *Biol.Psychiatry* 45.11 (1999): 1393-402.
94. Kawasaki, Y., et al. Structural brain differences in patients with schizophrenia and schizotypal disorder demonstrated by voxel-based morphometry. *Eur.Arch.Psychiatry Clin.Neurosci.* 254.6 (2004): 406-14.
95. Mohanty, A., et al. Neural mechanisms of affective interference in schizotypy. *J.Abnorm.Psychol.* 114.1 (2005): 16-27.

96. Kendler, K.S., Lieberman, J.A. and Walsh, D. The Structured Interview for Schizotypy (SIS): a preliminary report. *Schizophr.Bull.* 15.4 (1989): 559-71.
97. Johnstone, E.C., et al. Predicting schizophrenia: findings from the Edinburgh High-Risk Study. *Br.J.Psychiatry* 186 (2005): 18-25.
98. Rust, J. The Rust Inventory of Schizotypal Cognitions (RISC). *Schizophr.Bull.* 14.2 (1988): 317-22.
99. Lymer, G.K., et al. Brain-behaviour relationships in people at high genetic risk of schizophrenia. *Neuroimage.* 33.1 (2006): 275-85.
100. Johnstone E.C., Owens D. G. C. Hoare P. Gaur S. Spencer M. D. Harris J. Moffat V. Brearley N. Miller P. Lawrie S. M. Muir W. J. Schizotypal cognitions as a predictor of psychopathology in adolescents with mild intellectual impairement. *British Journal of Psychiatry* . 2007.
Ref Type: In Press
101. Turner, T.H. Schizophrenia and mental handicap: an historical review, with implications for further research. *Psychol.Med.* 19.2 (1989): 301-14.
102. Kraeplin, E. Dementia Praecox and Paraphrenia (Trans. R. M. Barcley, Ed. G. M. Robertson). Edinburgh: Livingstone, 1919.
103. Doody, G.A., et al. 'Pfropfschizophrenie' revisited. Schizophrenia in people with mild learning disability. *Br.J.Psychiatry* 173 (1998): 145-53.
104. Sanderson, T.L., et al. Neuroanatomy of comorbid schizophrenia and learning disability: a controlled study. *Lancet* 354.9193 (1999): 1867-71.
105. Meadows, G., et al. Assessing schizophrenia in adults with mental retardation. A comparative study. *Br.J.Psychiatry* 158 (1991): 103-05.
106. Hassiotis, A., et al. Prevalence and characteristics of patients with severe mental illness and borderline intellectual functioning. Report from the UK700 randomised controlled trial of case management. *Br.J.Psychiatry* 175 (1999): 135-40.
107. Bouras, N., et al. Schizophrenia-spectrum psychoses in people with and without intellectual disability. *J.Intellect.Disabil.Res.* 48.Pt 6 (2004): 548-55.
108. Chaplin, R., et al. The impact of intellectual functioning on symptoms and service use in schizophrenia. *J.Intellect.Disabil.Res.* 50.Pt 4 (2006): 288-94.
109. Moss, S., Prosser, H. and Goldberg, D. Validity of the schizophrenia diagnosis of the psychiatric assessment schedule for adults with developmental disability (PAS-ADD). *Br.J.Psychiatry* 168.3 (1996): 359-67.
110. Davidson, M., et al. Prevalence of psychiatric morbidity among remand prisoners in Scotland. *Br.J.Psychiatry* 167.4 (1995): 545-48.
111. Miller, P.M., et al. Childhood behaviour, psychotic symptoms and psychosis onset in young people at high risk of schizophrenia: early findings from the edinburgh high risk study. *Psychol.Med.* 32.1 (2002): 173-79.
112. Miller, P., et al. Schizotypal components in people at high risk of developing schizophrenia: early findings from the Edinburgh High-Risk Study. *Br.J.Psychiatry* 180 (2002): 179-84.
113. Achenbach T.L. Integrative Guide for the 1991 CBCL/4-118, YSR, and TRFProfiles. Burlington, VT. University of Vermont Department of Psychiatry, 1991.
114. Moorhead, T.W., et al. Voxel-based morphometry of comorbid schizophrenia and learning disability: analyses in normalized and native spaces using parametric and nonparametric statistical methods. *Neuroimage.* 22.1 (2004): 188-202.

115. Schachter, D.C., Pless, I.B. and Bruck, M. The prevalence and correlates of behaviour problems in learning disabled children. *Can.J.Psychiatry* 36.5 (1991): 323-31.
116. Wechsler, D. Wechsler Intelligence scale for Children III. 1992. New York, Psychological Corporation.
117. Wechsler, D. Wechsler Adult Intelligence Scale III. 1999. New York, Psychological Corporation.
118. Goldberg, D.P, et al. A standardized psychiatric interview for use in community surveys. *British Journal of Preventive and Social Medicine* 24 (1970): 18- 23.
119. Davidson, M., et al. Prevalence of psychiatric morbidity among remand prisoners in Scotland. *Br.J.Psychiatry* 167.4 (1995): 545-48.
120. Krawiecka, M., Goldberg, D. and Vaughan M. A standardized psychiatric assessment scale for rating chronic psychotic patients. *Acta Psychiatr.Scand.* 55.4 (1977): 299-308.
121. Kay, S.R., Fiszbein, A. and Opler, L.A. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr.Bull.* 13.2 (1987): 261-76.
122. Pruessner, J.C., et al. Volumetry of hippocampus and amygdala with high-resolution MRI and three-dimensional analysis software: minimizing the discrepancies between laboratories. *Cereb.Cortex* 10.4 (2000): 433-42.
123. Brierley, B., Shaw, P. and David, A.S. The human amygdala: a systematic review and meta-analysis of volumetric magnetic resonance imaging. *Brain Res.Brain Res.Rev.* 39.1 (2002): 84-105.
124. Watson, C., et al. Anatomic basis of amygdaloid and hippocampal volume measurement by magnetic resonance imaging. *Neurology* 42.9 (1992): 1743-50.
125. Szeszko, P.R., et al. Amygdala volume reductions in pediatric patients with obsessive-compulsive disorder treated with paroxetine: preliminary findings. *Neuropsychopharmacology* 29.4 (2004): 826-32.
126. DelBello, M.P., et al. Magnetic resonance imaging analysis of amygdala and other subcortical brain regions in adolescents with bipolar disorder. *Bipolar.Disord.* 6.1 (2004): 43-52.
127. Reiss, A.L., et al. An experiment of nature: brain anatomy parallels cognition and behavior in Williams syndrome. *J.Neurosci.* 24.21 (2004): 5009-15.
128. Schumann, C.M., et al. The amygdala is enlarged in children but not adolescents with autism; the hippocampus is enlarged at all ages. *J.Neurosci.* 24.28 (2004): 6392-401.
129. Brambilla, P., et al. Anatomical MRI study of borderline personality disorder patients. *Psychiatry Res.* 131.2 (2004): 125-33.
130. Lindauer, R.J., et al. Smaller hippocampal volume in Dutch police officers with posttraumatic stress disorder. *Biol.Psychiatry* 56.5 (2004): 356-63.
131. Rojas, D.C., et al. Hippocampus and amygdala volumes in parents of children with autistic disorder. *Am.J.Psychiatry* 161.11 (2004): 2038-44.
132. Makris, N., et al. Decreased absolute amygdala volume in cocaine addicts. *Neuron* 44.4 (2004): 729-40.
133. Niu, L., et al. Volume reduction of the amygdala in patients with schizophrenia: a magnetic resonance imaging study. *Psychiatry Res.* 132.1 (2004): 41-51.
134. Rosso, I. M., et al. Amygdala and hippocampus volumes in pediatric major depression. *Biol.Psychiatry* 57.1 (2005): 21-26.

135. Adolphs, R., D. Tranel, and T. W. Buchanan. Amygdala damage impairs emotional memory for gist but not details of complex stimuli. *Nat.Neurosci.* 8.4 (2005): 512-18.
136. Kalus, P., et al. The amygdala in schizophrenia: a trimodal magnetic resonance imaging study. *Neurosci.Lett.* 375.3 (2005): 151-56.
137. Tanskanen, P., et al. Hippocampus and amygdala volumes in schizophrenia and other psychoses in the Northern Finland 1966 birth cohort. *Schizophr.Res.* 75.2-3 (2005): 283-94.
138. Basso, M., et al. Apolipoprotein E epsilon4 is associated with atrophy of the amygdala in Alzheimer's disease. *Neurobiol.Aging* 27.10 (2006): 1416-24.
139. Yoshikawa, E., et al. Prefrontal cortex and amygdala volume in first minor or major depressive episode after cancer diagnosis. *Biol.Psychiatry* 59.8 (2006): 707-12.
140. Chey, J., et al. Medial temporal lobe volume of nondemented elderly individuals with poor cognitive functions. *Neurobiol.Aging* 27.9 (2006): 1269-79.
141. Horinek, D., et al. Amygdalar volume and psychiatric symptoms in Alzheimer's disease: an MRI analysis. *Acta Neurol.Scand.* 113.1 (2006): 40-45.
142. Zetzsche, T., et al. Amygdala volume and depressive symptoms in patients with borderline personality disorder. *Biol.Psychiatry* 60.3 (2006): 302-10.
143. Velakoulis, D., et al. Hippocampal and amygdala volumes according to psychosis stage and diagnosis: a magnetic resonance imaging study of chronic schizophrenia, first-episode psychosis, and ultra-high-risk individuals. *Arch.Gen.Psychiatry* 63.2 (2006): 139-49.
144. den Heijer T., et al. Use of hippocampal and amygdalar volumes on magnetic resonance imaging to predict dementia in cognitively intact elderly people. *Arch.Gen.Psychiatry* 63.1 (2006): 57-62.
145. Blumberg, H.P., et al. Preliminary evidence for persistent abnormalities in amygdala volumes in adolescents and young adults with bipolar disorder. *Bipolar.Disord.* 7.6 (2005): 570-76.
146. Barkataki, I., et al. Volumetric structural brain abnormalities in men with schizophrenia or antisocial personality disorder. *Behav.Brain Res.* 169.2 (2006): 239-47.
147. Teipel, S. J., et al. Comprehensive dissection of the medial temporal lobe in AD: measurement of hippocampus, amygdala, entorhinal, perirhinal and parahippocampal cortices using MRI. *J.Neurol.* 253.6 (2006): 794-800.
148. Goncalves Pereira, P.M., Oliveira, E. and Rosado, P. Apparent diffusion coefficient mapping of the hippocampus and the amygdala in pharmaco-resistant temporal lobe epilepsy. *AJNR Am.J.Neuroradiol.* 27.3 (2006): 671-83.
149. Dziobek, I., et al. The 'amygdala theory of autism' revisited: linking structure to behavior. *Neuropsychologia* 44.10 (2006): 1891-99.
150. Vermetten, E., et al. Hippocampal and amygdalar volumes in dissociative identity disorder. *Am.J.Psychiatry* 163.4 (2006): 630-36.
151. Kates, W.R., et al. Temporal lobe anatomy and psychiatric symptoms in velocardiofacial syndrome (22q11.2 deletion syndrome). *J.Am.Acad.Child Adolesc.Psychiatry* 45.5 (2006): 587-95.
152. Munson, J., et al. Amygdalar volume and behavioral development in autism. *Arch.Gen.Psychiatry* 63.6 (2006): 686-93.

153. Debbane, M., et al. Hippocampal volume reduction in 22q11.2 deletion syndrome. *Neuropsychologia* 44.12 (2006): 2360-65.
154. Convit, A., et al. MRI volume of the amygdala: a reliable method allowing separation from the hippocampal formation. *Psychiatry Res.* 90.2 (1999): 113-23.
155. Kinnear, P.R and Gray, C.D. SPSS 14 Made Simple. Psychology Press Ltd, 2006.
156. Watson, C., et al. Specificity of volumetric magnetic resonance imaging in detecting hippocampal sclerosis. *Arch.Neurol.* 54.1 (1997): 67-73.
157. Arndt, S., et al. Problems with ratio and proportion measures of imaged cerebral structures. *Psychiatry Res.* 40.1 (1991): 79-89.
158. Mathalon, D.H., et al. Correction for head size in brain-imaging measurements. *Psychiatry Res.* 50.2 (1993): 121-39.
159. Bogerts, B., et al. Hippocampus-amygdala volumes and psychopathology in chronic schizophrenia. *Biol.Psychiatry* 33.4 (1993): 236-46.
160. Spear, L.P. The adolescent brain and age-related behavioural manifestations. *Neuroscience Behavioural Review* 24 (2000): 417-463.
161. Nelson, E.E., et al. The social re-orientation of adolescence: A neuroscience perspective on the process and its relation to psychopathology. *Psychological Medicine* 35 (2005): 163-174.
162. Whittle, S., et al. Prefrontal and amygdala volumes are related to adolescents' affective behaviours during parent-adolescent interactions. *Proceedings of the National Academy of Sciences* 105.9 (2008): 3652-3657.
163. Giedd, J.N., et al. Quantitative MRI of the temporal lobe, amygdala, and hippocampus in normal human development: ages 4-18 years. *Journal of Comparative Neurology* 366.2 (1996): 223-230.
164. Chen, K.N. et al. Cross-sectional study of abnormal amygdala development in adolescents and young adults with bipolar disorder. *Biological Psychiatry* 56 (2004): 399-405.
165. Sanderson, T. L., et al. Correlations between clinical and historical variables, and cerebral structural variables in people with mild intellectual disability and schizophrenia. *J.Intellect.Disabil.Res.* 45.Pt 2 (2001): 89-98.
166. Owens, D.G., et al. Pathogenesis of schizophrenia: a psychopathological perspective. *Br.J.Psychiatry* 186 (2005): 386-93.
167. Cutting, JC. Descriptive Psychopathology. Schizophrenia. Ed. SR Hirsch and Weinberger D. London: Blackwell, 2003. 15-24.
168. DeBellis, et al. A pilot study of amygdala volumes in pediatric generalized anxiety disorder. *Biol.Psychiatry* 48.1 (2000): 51-57.
169. Xue, Y. Neighborhood residence and mental health problems of 5- to 11-year olds. *Archives of General Psychiatry* 62 (2005): 554-563.
170. Johnstone, E.C., et al. Companion to Psychiatric Studies. Seventh Edition ed. Edinburgh: Churchill Livingstone, 2004.
171. Kurachi, M. Pathogenesis of schizophrenia: Part II. Temporo-frontal two-step hypothesis. *Psychiatry Clin.Neurosci.* 57.1 (2003): 9-15.
172. Sheitman, B.B., et al. Are the negative symptoms of schizophrenia consistent with an autistic spectrum illness? *Schizophr.Res.* 69.1 (2004): 119-20.
173. Konstantareas, M.M. and Hewitt, T. Autistic disorder and schizophrenia: diagnostic overlaps. *J.Autism Dev.Disord.* 31.1 (2001): 19-28.

174. Rumsey, J. M., Andreasen, N.C. and Rapoport, J.L. Thought, language, communication, and affective flattening in autistic adults. *Arch.Gen.Psychiatry* 43.8 (1986): 771-77.
175. Aylward, E.H., et al. MRI volumes of amygdala and hippocampus in non-mentally retarded autistic adolescents and adults. *Neurology* 53.9 (1999): 2145-50.
176. Pierce, K., et al. Face processing occurs outside the fusiform 'face area' in autism: evidence from functional MRI. *Brain* 124.Pt 10 (2001): 2059-73.
177. Spencer, M.D., et al. Grey matter correlates of early psychotic symptoms in adolescents at enhanced risk of psychosis: a voxel-based study. *Neuroimage.* 35.3 (2007): 1181-91.
178. Hall, J., et al. Emotional memory in schizophrenia. *Neuropsychologia* 45.6 (2007): 1152-59.
179. Grossberg, S. The imbalanced brain: from normal behavior to schizophrenia. *Biol.Psychiatry* 48.2 (2000): 81-98.
180. Kapur, S., et al. From dopamine to salience to psychosis - linking biology, pharmacology and phenomenology to psychosis. *Schizophrenia Research* 79 (2005): 59-68.
181. Broome, M.R. What causes the onset of psychosis? *Schizophrenia Research* 79 (2005): 23-34.
182. Berridge, K.C. et al. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res. Rev.* (1998) 28: 309-369.
183. Laurelle, M., et al. Increased dopamine transmission in schizophrenia. *Biological Psychiatry* 46 (1999): 56-72.
184. Phillips, A.G. Amygdalar control of the mesocorticolimbic dopamine system: parallel pathways to motivated behaviour. *Neuroscience and Behavioural Reviews* 27 (2003): 543-554.
185. Grace, A.A. Gating of information flow within the limbic system and the pathophysiology of schizophrenia. *Brain Res. Rev.* 31 (2000): 330-341.
186. Duvernoy, H. The Human Brain: Surface, Three-Dimensional Sectional Anatomy With MRI, and Blood Supply. New York: Sprimger-Verlag, 1999.